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EFFECTS OF NURSERY-ENVIRONMENT CONDITION ON HABITAT USE, GROWTH, SURVIVAL AND ENDOCRINE PHYSIOLOGY DURING LARVAL SETTLEMENT IN THE RED DRUM (*SCIAENOPS OCELLATUS*)

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by

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Dedication

A mi familia.

Este trabajo va dedicado a mis padres, Antonio y Pastora, sus años de apoyo, sacrificio y confianza han puesto los cimientos que han hecho posible esta meta. Esta dedicatoria estaría incompleta sin Maria del Carmen, su constante aliento, amor, sacrificio y compañía, han hecho estos años verdaderamente inolvidables. Diego y Cecilia, me habéis contagiado de vuestra alegría y hecho ver fácil lo imposible. Gracias a todos.

To my family.

To my parents, Antonio and Pastora, their years of support, sacrifice and trust have laid down the foundations that made this goal possible. This dedication would be incomplete without Maria del Carmen, her constant support, love, sacrifice and companion have made these years truly unforgettable. Diego and Cecilia, you have infected me with your happiness and made easy the impossible. Thank you all.

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Settlement in red drum (*Sciaenops ocellatus*) involves vulnerable early-life stages that rely upon fast growth and reduced mortality within seagrass nursery areas. Environmental parameters fluctuated widely in seagrass as a result of daily cycles or weather systems. Major diel variability was found in temperature (amplitude: 3-7 ºC) and dissolved oxygen (DO) (range: 2.9-17.5 mg O_2 l^{-1}; hypoxia-hyperoxia). Hypoxia events were regularly observed, especially at the core of seagrass beds where they lasted for longer periods compared to deep-edge areas. Cold fronts were associated with important and fast changes in temperature and salinity. This study describes patterns of use of the nursery during settlement, as well as growth and mortality estimates during subsequent

recruitment. The ratio between instantaneous growth in weight and mortality coefficients (G':Z) was used as a criterion to address the value of seagrass areas for young fish. In the laboratory environmentally realistic temperature and DO fluctuations did not affect growth or survival of larvae. I detected an early activation of thyroid and interrenal glands during the yolk-sac phase and a second activation of the thyroid gland during transformation into juveniles which was coincident with settlement. Settlement-size larvae exposed to handling expressed stress-related cortisol changes. However, no such increase was induced by dawn-hypoxia or normal diel temperature cycles. This study detected a pulsed supply of settlers from early September to late October coupled with high mortality rates, suggesting that population size largely depends on supply and larval mortality immediately after settlement. Red drum larvae settle at the edge and core seagrass, but accumulate at the core seagrass. Mortality was substantial and variable, determining the value of G':Z. No seasonal trends in G':Z were observed and probably most cohorts contributed to recruitment. Cages stocked at edge and core areas with hatchery-reared larvae failed to demonstrate habitat differences. Caged and wild fish grew at similar rates suggesting that cultured red drum can be used to estimate growth rates of wild counterparts during settlement. I suggest that edge seagrass areas are very important in determining successful settlement in this species since they provide the first and crucial contact with the nursery habitat.

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Chapter 1: General Introduction

Marine fisheries are a natural resource of substantial economic and social importance to coastal areas worldwide. Numerous fish populations are declining which leaves empty niches that result in permanent changes in natural communities (Caddy 1993; Myers and Worm 2003). Anthropogenic impacts such as overexploitation of stocks, pollution, and habitat deterioration are recognized to be in large part responsible for these declines and community changes (Holt 2002). Ensuring the future health of fish populations and their use will largely depend on our ability to identify and protect essential fish habitats from further degradation. Identification of essential fish habitats requires the evaluation of fish dependency in particular habitats, but more importantly a broad understanding of fish physiological responses to site-specific variability that make some habitats, and sites within habitats, more valuable than others (Beck *et al.* 2001; Baskin *et al.* 2003).

Common to most marine fish species is the production of vast numbers of small pelagic larvae. This kind of reproduction is favored in environments where the survival of the offspring depends on a number of stochastic processes that cannot be precisely anticipated (Winemiller and Rose 1993). Most of these larvae will die within a brief period (Houde 2002), however some variable proportion will reach suitable habitats where they will grow and develop to eventually join the adult stock. In this reproductive strategy, the larval stage maintains the integrity of the stocks and determines the level of replacement for the next generation (i.e. recruitment). It is now widely accepted that fluctuations in population size results from the combination of high fecundities and differential mortality during the early life (Hjort 1914, Houde 1987). More generally stated, variable recruitment arises from differential growth and mortality rates at variable

temporal and spatial scales (Cushing 1975; Lasker 1978; Houde 1987, Cowan *et al.* 1996). However, the causes of the large and often inexplicable interannual variability are still unknown.

Many marine fishes exhibit life-history variations in which separate nursery habitats are used during the early life period. Settlement of early life stages to benthic nursery habitats is often linked to variability in year-class recruitment (Underwood and Fairweather 1989; Sale 1990; Heath 1992; Searcy and Sponaugle 2001). A suite of ecological and developmental processes operates during this transition and during this use of the nursery grounds. Often these nursery areas are located within the boundaries of the oceans and in estuarine systems where patterns of productivity favor growth of the young. The ratio between instantaneous growth rate in weight (G') and mortality rate (Z) has been used to evaluate larval cohort performance. Estimates of survival potential (Z:G') or growth potential (G':Z) of larval cohorts during the postsettlement period can serve as an index with which to compare nursery habitat quality and ultimately habitat value for the early stages of fishes (Houde 1987 and 1989). G':Z is used here to investigate differences in habitat quality. Varying biotic, abiotic and landscape characteristics of the nursery can create site- and time-specific environments that can translate into differential supply, growth or survival of larvae, leading to variable recruitment (Sogard 1992; Beck *et al.* 2001). An appreciation of the physical and biological factors controlling settlement and subsequent use of the nursery habitat is thus essential to understand and predict the dynamics of marine fish populations.

The organism used in this study, red drum (*Sciaenops ocellatus*), is an economically important species commonly found along the Atlantic (up to 40ºN; Delaware Bay) and Gulf of Mexico Coasts of the United States. Well-developed captive spawning (Arnold 1988, Thomas *et al.* 1995) and larval rearing techniques (Holt *et al.*

1990), along with a relatively good knowledge of their early life history (Holt *et al.* 1983; Rooker and Holt 1997; Rooker *et al.* 1997, 1998b, 1999; Holt and Holt 2000) and high recreational value make red drum an excellent model for studies that couple physiological and ecological research for a valuable fishery. Red drum spawn daily from August through October along shallow coastal waters of the northern Gulf of Mexico. Eggs and larvae remain in the plankton until they reach 4-6 mm standard length (SL) at which time they are transported through coastal inlets to estuaries where seagrass beds and marsh edges serve as settlement and primary nursery habitat (Holt *et al.*1983, 1989; Rooker *et al.*1997, 1998b and 1999; Stunz and Minello 2001). Red drum settle between 4 and 11 mm SL (peak settlement size is 6-8 mm SL) (Herzka *et al.* 2002). There are, however, relative low catches in the 6-8 mm size class in seagrass collections (Rooker *et al.* 1999, Herzka *et al.* 2002). These sizes are also absent from plankton surveys (Holt *et al.* 1989; Holt and Holt 2000). To date, no indications of possible alternate habitats for these size classes have been found and no information is available on the use of deeper (1.2 m) areas of the seagrass during settlement.

Seagrass-dominated landscape features discrete patches of often monospecific vegetation ranging in size from one to several thousand of square meters separated by bare sediment areas. Seagrass beds can be found worldwide in the shallow subtidal and intertidal regions along tropical, subtropical and temperate coastal waters (Robins and Bell 2000). The lower (deepest) boundary is traditionally linked to light and/or substrate nutrient availability and the upper (shallower) boundary to desiccation. Seagrass meadows vary from relatively deep, patchy habitat at the deep edge to shallow, more uniformly vegetated habitat at the core of the meadows. Substantial fluctuation in temperature, salinity, dissolved oxygen (DO) or nutrients are distinctive features of estuarine nursery environments (Hubertz and Cahoon. 1999; Robbins and Bell 2000).

Environmental conditions fluctuate daily in shallow estuarine areas as a result of day:night and tidal cycles. Temperature cycles and DO fluctuations between hypoxia and hyperoxia over the diel cycle have been reported in estuarine systems (D'Avanzo & Kremer, 1994; Ziegler and Benner 1998; Beck and Bruland 2000). Diel biogeochemical cycles linked to patterns of oxygen availability in seagrass sediments have also been reported (Lee and Dunton 2000). This implies potential for diel changes in oxygen availability and temperature regimes in seagrasses. Depth-related microhabitat differences within seagrass meadows may result in differential habitat utilization that may affect growth and/or survival during settlement and therefore determine differences in habitat quality (Gibson 1994; Beck *et al.* 2001; Basking *et al.* 2003; Holbrook and Schmitt 2003). The range of diel environmental fluctuation within red drum nursery areas and the effects of this variability on red drum larvae are unknown.

Larval fish experience dramatic ontogenetic changes and explosive growth rates as they transition from a poorly differentiated state at hatching to the adult-like juvenile (Blaxter 1986; Fuiman *et al.* 1998). The regulation of growth and ontogeny, and the addition of new or improved physiological competence follow a well-defined and genetically-programmed development in which thyroid and steroid hormones play an essential role (Brown and Bern 1989; de Jesus *et al.*1990 and 1991; Inui *et al*. 1994; Tanaka *et al.* 1995). In most cases hormones show diel fluctuations that respond to both endogenous and environmental cues (Leatherland 1982; Pickering and Pottinger 1983; Leiner and Meier 1992; Leiner and MacKenzie 2003). Extreme environmental condition may cause cortisol-mediated stress responses, and reduced growth (Andersen 1991; Van Weerd, and Komen, 1998). Furthermore ontogenetic hormonal surges are related to “preadaptive” (Specker 1988) changes in fish physiology and body form that prepare organisms to deal with habitat transitions (Specker 1988; Tagawa and Hirano 1990;

Tanaka *et al*. 1991; Tanaka 1995; Perez *et al.* 1999). Settlement usually coincides with the final transformation into juveniles, a process that is regulated by thyroid hormones and cortisol. The effects of environmental factors on hormone production and the interaction with ontogeny are poorly understood in fish larvae. Understanding the interconnection between environmental conditions and endocrine function, and their effects on growth and survival in the nursery may help to identify sources of recruitment variability.

The aim of these studies was to: (1) describe naturally occurring environmental rhythms within red drum nursery habitats and determine the effects of temperature and DO cycles on growth and mortality in laboratory studies under simulated natural nursery conditions and in caging experiments, (2) describe ontogenetic and diel profiles of thyroid hormones and cortisol during settlement and determine the cortisol responsiveness of red drum larvae to several environmental stimuli, (3) determine diel and seasonal patterns of distribution and abundance of red drum larvae in a representative Texas bay, and estimate growth and mortality rates from red drum surveys to calculate recruitment potential of natural cohorts after settlement into the nursery, and (4) evaluate the use of caging experiments as a tool to assess growth and mortality estimates of natural cohorts.

The overall hypotheses during the study are as follows:

Ho_1: Microhabitat-related differences in environmental conditions in space and time do not affect recruitment potential.

Ho_2: Settling red drum larvae do not select particular microhabitats within a homogeneous seagrass meadow.

Ho_3: Ontogenetic changes in hormone profiles are not correlated with settlement events in this species.

Experimental questions associated with these hypotheses include the following examples: Does recruitment potential vary in space and time? Are there superior areas within seagrasses? What are the environmental attributes of those areas? Does the endocrine development of the larvae correlate with ontogenetic landmarks in the early life of the species?

Chapter 2: Environmental variability in seagrass meadows: Effects of nursery environment cycles on larval red drum (*Sciaenops ocellatus*) growth and survival

ABSTRACT

In their early larval stages, red drum migrate through coastal inlets and settle into shallow seagrass meadows within estuaries. This study describes environmental rhythms in red drum nursery habitats and evaluates their role in larval growth and survival to determine nursery habitat quality. Well-defined diel cycles were observed in temperature (amplitude: 3 to 7 ºC) and dissolved oxygen (DO) (range: 2.9-17.5 mg O_2 l^{-1}), while sporadic fronts dropped temperatures 6-10 ºC in 24-72 h. Groups of settlement and post-settlement larvae (3.9-17.3 mm SL) were exposed in the laboratory to cycles of temperature and DO, and to combined temperature and DO diel cycles and then compared to fish grown in constant conditions (control). Relative to controls, growth was significantly reduced only in DO cycles with prolonged exposure (>34.2% total time) to hypoxia. Survival was similar in all treatments. Fish previously exposed to temperature cycles grew faster and had higher food intake than control fish in response to water cooling during simulated cold fronts. Fish exposed to DO cycles maintained greater food intake but grew at a similar rate to control fish. These results indicate that: 1) diel cycles impart a physiological advantage to red drum larvae, 2) hypoxia in oscillating environments can affect growth, 3) zero growth is predicted at 8.7 ºC, and 4) field measurements of environmental characteristics made at a frequency of once per day may be inadequate for predicting fish growth. Identification of environmental conditions and mechanisms that resulted in best growth are necessary to assess nursery value for red drum.

INTRODUCTION

Recruitment is an issue central to fish population dynamics. Fish nursery areas are thought to play a critical role in determining adult population size by influencing recruitment success (Underwood and Fairweather 1989; Sale 1990). Red drum (*Sciaenops ocellatus*) spawn in coastal waters of the Gulf of Mexico from late August to October (Peters and McMichael 1987). Eggs hatch in less than 24 h and after 2-3 weeks in the plankton, the larvae settle to seagrass beds and marsh edges within estuaries (Holt *et al.* 1983; Rooker and Holt 1997; Rooker *et al.* 1999; Stuntz *et al*. 2002). In the Aransas Estuary, seagrass beds serve as settlement and primary nursery habitat for young red drum (Rooker and Holt 1997, Rooker *et al.* 1998). Rapid larval growth during the extremely vulnerable larval period greatly increases the probability of survival (Pepin 1989; Fuiman and Magurran 1994; Cowan *et al.* 1996), and can result in order-of-magnitude changes in recruitment (Houde 1989 and 2002).

Many species of marine fish exhibit an estuarine dependent life cycle in which spawning takes place offshore and recruitment to estuarine habitat occurs during early life. Structured estuarine habitats can provide a plentiful food supply as well as shelter from predators. However, shallow estuarine habitats also may exhibit substantial fluctuations in environmental parameters such as salinity, temperature and dissolved oxygen (Hubertz and Cahoon 1999; Robbins and Bell 2000) to which newly recruited individuals may be exposed. Local variations in temperature, dissolved oxygen (DO) and salinity may be considerable as a result of diel and tidal cycles and stochastic atmospheric events (Summer *et al.* 1997; Ziegler and Benner 1998). Diel variations in temperature of 3 to 5 ºC have been reported in shallow estuarine environments (Beck and Bruland, 2000). Similarly, water cooling associated with atmospheric frontal systems (fronts) is

also common during red drum settlement season (Brown 2000, 2004). Dissolved oxygen concentrations (DO) may be large in shallow subtropical seagrass meadows due to photosynthesis and respiration of benthic communities (Ziegler and Benner 1998; Beck and Bruland 2000). Temperature and DO fluctuations in seagrass beds are possibly the most important abiotic factors controlling growth during the larval period (Fry 1947 and 1971), and can therefore play an important role in recruitment.

A limited number of studies have examined the relationship between natural fluctuations in abiotic parameters and growth during the early life stages of marine fishes (De Silva and Tytler 1973). Although the effects of temperature and DO concentrations on fish growth and survival have been studied intensively and summarized in numerous reviews (e.g. Fry, 1971; Blaxter 1992; Rombough, 1988), very few studies have examined the effect of fluctuating environmental conditions on fish growth and distribution (Duthie and Houlihan, 1982; Pihl *et al.* 1991; Diaz and Rosenberg 1995; Thetmeyer *et al.* 1999). Taylor and Miller (2001) reported a reduction in growth of southern flounder (*Paralichthys lethostigma*) exposed to periods of nocturnal hypoxia (2.8 mg O_2 l^{-1}). Simulations of juvenile red drum growth performance made under time-varying environmental regimes indicate that given an unrestricted food supply, growth may be much faster under diel-cycling regimes than under constant optimal temperature and DO regimes (Neill *et al*. 2004). Neill *et al.* (2004) related faster growth under variable conditions to a directive response to low dissolved oxygen concentrations that resulted in an increased metabolic capacity during the normoxic regime. In contrast to the constant environmental conditions in which fish are typically held in the laboratory, diel cycles of environmental parameters may contain intervals of nearly ideal conditions for growth that compensate for time spent under less optimal conditions. This may result in an overall greater metabolic scope for growth.

The aim of the present study was two-fold: 1) to describe the naturally occurring environmental rhythms in temperature and DO (range and patterns of variation) within red drum nursery habitat during the settlement period, and 2) to simulate these temperature and DO cycles in the laboratory to evaluate their effects on larval growth and survival.

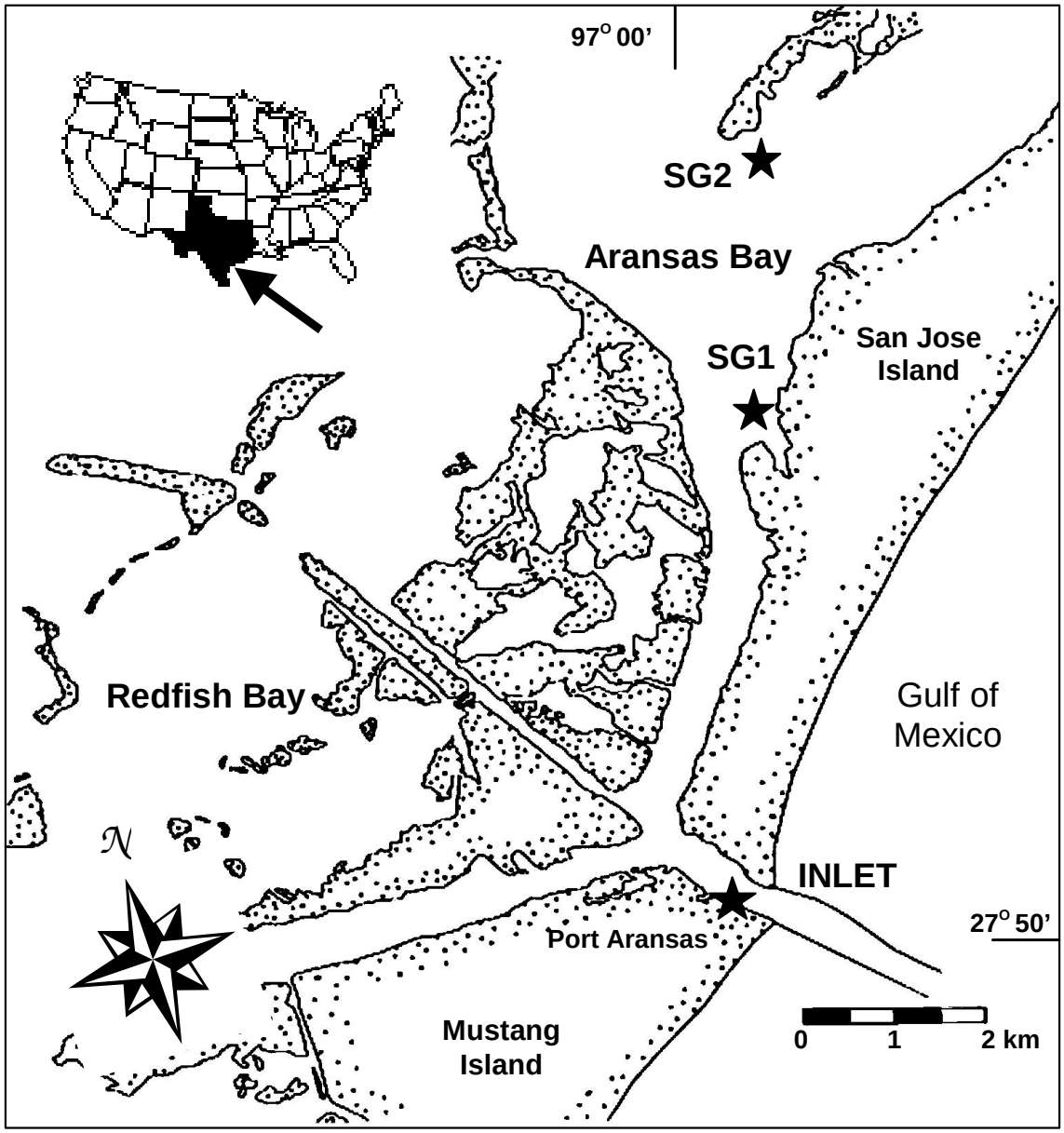
MATERIALS AND METHODS

Environmental surveys

Characterization of environmental parameters

Environmental conditions were surveyed at three locations in the Aransas Bay system near the only inlet to the estuary during the fall of 2000 and 2001. Two stations were located in shallow seagrass beds (SG1 and SG2; Fig. 2.1). Newly settled red drum larvae have been collected previously at these sites (Rooker and Holt 1997; Rooker 1999; Herzka 2001). A third station (INLET) was located near Port Aransas in the inlet linking the estuary to the Gulf of Mexico where planktonic larvae are regularly collected (Holt and Holt 2000) (Fig. 2.1). Temperature, DO, pH, conductivity, turbidity (turbidity in year 2000 only) and water depth in the seagrass stations were recorded at 15- or 30-min intervals with multiparameter water quality data sondes (YSI Incorporated, OH, USA) placed within the seagrass canopy. Data sonde readings were compared with discrete observations made throughout each deployment to assess data quality. Environmental data from the INLET station, as well as air temperature, were obtained from an automated monitoring system at the Pier Laboratory of the University of Texas Marine Science Institute (UTMSI), which is located in the inlet. All studies coincided with the peak period of larval red drum settlement to seagrass meadows (October-November).

Figure 2.1. Map of the study site. Stars mark the location of the studied seagrass meadows and the inlet station. The arrow marks the general study area in the south Texas coast.



Seasonal trends in environmental parameters and the effect and periodicity of atmospheric frontal systems were assessed in 2002 and 2003. Data sondes were deployed continuously at two locations within SG1. The sondes were placed toward the deepest edge and middle of the seagrass meadow for 7 and 11 weeks in 2002 and 2003 respectively. Data sondes were checked and data downloaded weekly to ensure proper working condition, calibrate sensors and prevent loss of data.

Spatial and temporal variability within seagrass meadows

Fine-scale spatial variability in environmental characteristics within a seagrass bed (SG1) was evaluated during the 2001 settlement season. Data sondes were deployed along a transect from the shallowest to the deepest part of the seagrass meadow during two separate trips and recovered after 11 and 14 d. In the first deployment, data sondes were placed at the deepest edge and at the shallow bank (the core) of the meadow. The second deployment started 3 days later, and a second core and a shallow marsh-edge location were surveyed. Both deployments coincided in time for 10 days.

Effects of diel temperature and DO fluctuations on growth and survival of settlement-sized red drum

Rearing and experimental set up

Fertilized eggs were obtained through the manipulation of temperature and photoperiod of captive broodstocks (Arnold, 1988) held at the UTMSI Fisheries and Mariculture Laboratory and at the CP&L Marine Development Center, Texas Parks and Wildlife Department in Corpus Christi, TX. Eggs were treated with 0.1% formalin for 30 min and placed in 150 l tanks (50-100 eggs l^{-1}) at 26-27 ºC, 27-31 psu in well-aerated filtered seawater. The tank was put on a flow-through system (25 ml min^{-1}) following stocking of eggs. Hatching rates for each tank were estimated after ca. 20 h of incubation

based on the average number of live larvae in five 100 ml water samples. Only spawns with hatching rates greater than 95% were used. Starting 3 days post hatching (dph), first feeding larvae were provided daily with rotifers enriched with live cultures of *Isochrysis galbana* (UTEX LB 2307) as well as a formulated larval feed (Kyowa B 250µm; Kyowa Hakko Kogyo Co., Ltd. Tokyo, Japan) (Holt 1993). When larvae reached 3 mm standard length (SL; 8-10 dph), they were transferred to 350 l flat-bottom circular tanks (20-30 larvae l^{-1}) equipped with a biological filter. The water-exchange rate was 60-120 ml min^{-1}. Larvae were weaned onto the formulated feeds 1-3 days after transfer to the larger tanks. Formulated feed of various sizes (Kyowa B, 400-1700 µm) were used during most experiments. However, for fish reared in 2003 and 2004, brine shrimp (*Artemia* sp.) enriched overnight on *I. galbana* were used in addition to a formulated feed (Proton 2 INVE AQUACULTURE Inc., Ogden, UT).

Upon reaching 4-5 mm SL (16-19 dph), red drum larvae were randomly assigned to six 120 l experimental tanks at a density of approximately 6 larvae l^{-1} and allowed to acclimate for 24 h. The six tanks were then divided into two clusters of three replicate tanks. Each cluster was connected to a separate 150 l reservoir to create two independent recirculating systems (Fig. 2.2). One system was assigned to one of six diel environmental fluctuation treatments (see below) and the other was maintained at constant conditions (control).

Six experimental treatments were designated (Table 2.1): two with oscillating temperatures (TEMP1 and TEMP2), three with oscillating DO (DO1, DO2 and DO3) and a combined oscillating temperature and DO treatment (CYCLES). TEMP1 and TEMP2 were chosen to reflect low and high amplitude diel cycles (27.0 ± 1.5 and 27.1 ± 3.0 ºC, respectively). The three oscillating DO treatments were designed to result in various patterns of diel oxygen availability: DO1 was hypoxic-normoxic (dawn-noon

respectively) (2.8-6.1 mg O_2 l^{-1}), DO2 was hypoxic-hyperoxic, (2.1-12.4 mg O_2 l^{-1}) and DO3 was hypoxic-hyperoxic (3.5-12.8 mg O_2 l^{-1}). All tanks subjected to oscillations in DO and their respective controls were kept at constant temperature (27.0 ± 0.3 ºC). The CYCLES treatment involved oscillating temperature (27 ± 2 ºC) and DO (3.7-12.8 mg O_2 l^{-1}). Growth and survival of fish subjected to the varying experimental treatments were compared to larvae held at uniform temperatures (20-27 ± 0.3 ºC) in constantly well-oxygenated water (6.1 ± 0.7 mg O_2 l^{-1}, Control).

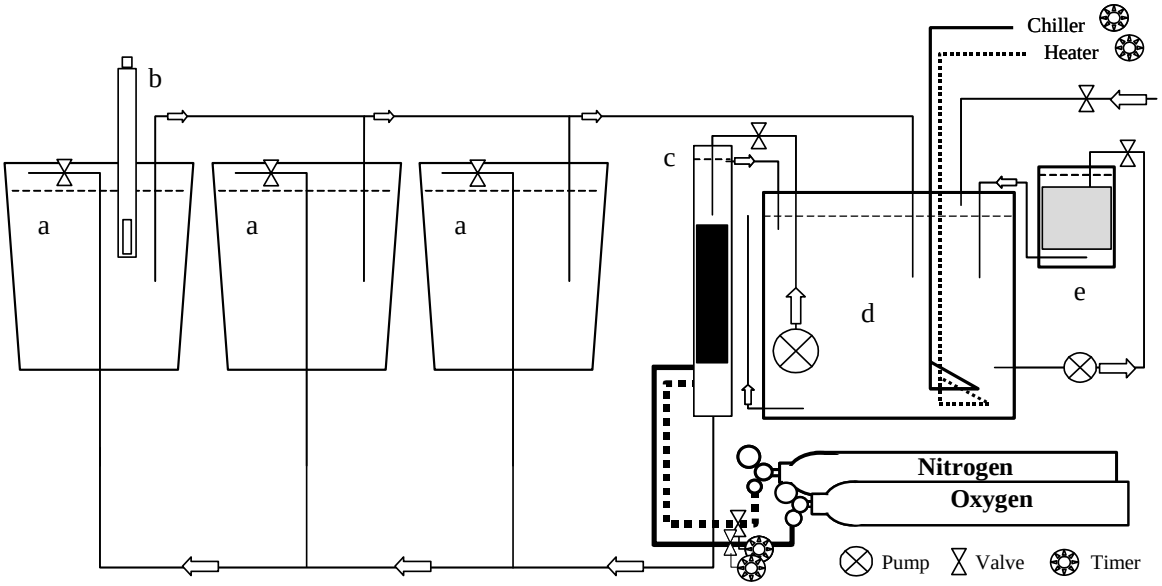
Temperature cycles were imposed using timer-controlled heaters (total of 1500 watts) and a water chiller (3,080 BTU h^{-1}) connected to the 150 l reservoir (Fig. 2.2). A countercurrent gas depletion column was placed between the reservoir and experimental tanks to generate the desired fluctuating DO conditions (Fig. 2.2). Timer-controlled solenoid valves controlled the timing of gas injection into the column. DO levels were established through the injection of nitrogen or oxygen into the bottom of the column. Predictable fluctuating regimens were obtained by trial-and-error manipulations of timer's switching times (cycle timing), and intensity of heat and gas injection (cycle amplitudes). Water was recirculated through each system 1.2 times h^{-1} to ensure a homogeneous environment among the three replicate tanks within a treatment. Temperature, DO, pH and salinity were recorded in at least one tank from each treatment at 15- or 30-min intervals by a multiparameter water quality data sonde. Approximately 10% of the total water volume was exchanged daily. Photoperiod was 12L:12D. Experiments lasted until the fish reached 30 mm SL. Each treatment was repeated twice except DO2 in which three runs were conducted.

Table 2.1. Experimental conditions in the treatment tanks. Values represent means ± SE.

Treatment	Temperature (°C)		DO (mg O_2 l^{-1})	
	min (dawn)	max (noon)	min (dawn)	max (noon)
TEMP1	25.5	28.5	constant (6.7 ± 0.6)	
TEMP2	24.1	30.0	constant (6.2 ± 0.7)	
DO1	constant (27.1 ± 0.3)		2.0	6.1
DO2	constant (27.3 ± 0.2)		1.9	12.4
DO3	constant (26.8 ± 0.5)		3.7	14.1
CYCLES [(1)]	20-27.3 ± 3.2		2.1-3.7	14.2

[(1)] Combined high amplitude temperature cycles with DO3-type DO oscillation.

Figure 2.2. Experimental rearing system. a. experimental tanks (120Lx3), b. multiparameter water-quality data logger, c. gas depletion column, d. reservoir (150L), e. biological filter. The arrows indicate the direction of the flow.



Sampling

A total of 20-25 fish from each tank was sampled at regular intervals throughout each experiment. The combined temperature and DO trial was sampled only once at the end of the experiments and lasted only for 9 to 12 days to allow more precise mortality calculations. All fish were anesthetized with 0.1% tricaine methanesulfonate (MS222) and the SL measured from the tip of the snout to the end of the notochord or caudal peduncle. All measurements were made to the closest 0.1 mm using SigmaScan Pro 4.01.003 (SPSS Inc., Chicago, IL) image-analysis software.

Effect of storm-related cooling

Fish conditioning and experimental set up

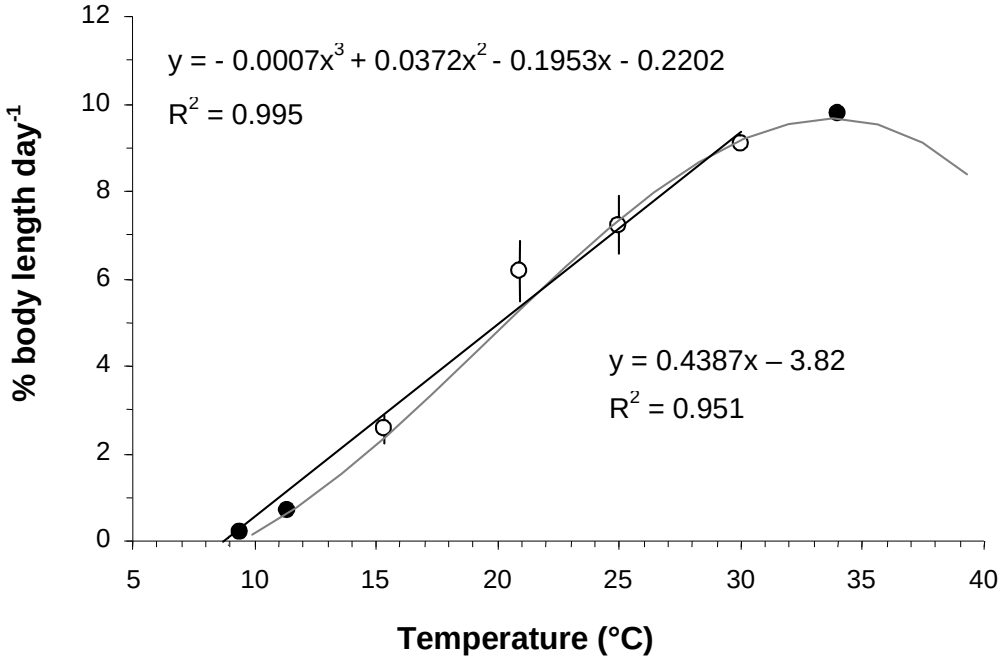
Fish were conditioned to an oscillating temperature or DO environment for 2 to 3 weeks and then subjected to a simulated strong cold front. The conditioning treatments were chosen by identifying the cycling conditions that did not result in growth differences relative to control fish to avoid confounding effects of different body size after the conditioning period. To simulate the rapid decrease in temperature associated with the onset of a cold front, three groups of 18 fish per treatment (DO or temperature-conditioned fish) were transferred to 6 enclosures (height 0.5 m, diameter 0.3 m, 700 µm mesh) placed within 350 l cylindrical tanks. Water temperature was decreased by 10 ºC (27 to 17 ºC) over a 24 h period. Temperature was maintained at 17-18 ºC for 2 days, raised to 27 ºC on day 3 and held at that temperature until the end of the experiment 3 days later (day 6). Temperature, DO, salinity and pH were monitored at 15- 30-min intervals as described previously. Food levels were adjusted daily to ensure that fish ate to satiation. Every morning all enclosures were carefully siphoned. The particulate food not consumed was collected and dried at 60 ºC for 36 h. Food consumption was estimated

by subtracting the food recovered (corrected for leaching, 24-h water stability test) from the total amount of food provided. Fish were measured 5 days before the beginning of the experiment while still in the conditioning tanks, on the day of transfer to the 350-l tanks, at the end of the cold phase and of the experiment. To minimize handling stress during length measurements, enclosures were lifted partially out of the water and placed in a 0.2-m deep tray prior to their removal from the tanks. Fish were then quickly anesthetized with MS222 (0.03 mg l^{-1}), photographed and returned to a clean mesocosm within the tank. Red drum larvae typically recovered from the anesthesia within 1 minute. Standard length measurements were performed using the image analysis system described previously.

Growth and survival

Minor temperature differences between treatments were taken into account by using effective degree-days to compare among treatments (DD_{eff}) (Fry, 1971; Kamler, 1992). DD_{eff} were calculated by subtracting a threshold temperature (i.e. biological zero, T_0) from the daily average temperature before computing thermal sums. Conceptually, the biological zero represents the temperature at which red drum larvae cease to grow. It can be estimated as the x-intercept in the linear regression of growth rate on temperature. To estimate T_0, two groups of red drum (SL = 11.1 and 11.0 mm) were grown in triplicate at approximately five-degree intervals from 9.4 to 34.1 ºC. The relative change in growth rate (% of initial body size) indicated a decrease at both extremes of the tested temperature range (Fig. 2.3). Hence, the linear regression was restricted to the growth rates obtained within the linear area of the relationship (11-30 ºC). T_0 was 8.7 ºC (R^2=0.95).

Figure 2.3. Growth rate of larval red drum at different experimental temperatures. The black line is a least-squares linear regression fitted to the central section (open circles). The grey line is a third order polynomial regression fitted to all data. Regression equations are given.



Instantaneous growth coefficients (G) were calculated from an exponential growth model:

$$G=[Ln(SL_t)-Ln(SL_0)]/ DD_{eff}$$

where SL_0 and SL_t are the standard lengths at the beginning and end of the experimental interval and DD_{eff} represents effective degree-days.

Percent survival (S) was calculated at the end of each trial from the equation

$$S=100\% [(N_f+N_s)/N_0]$$

where N_f is the number of remaining fish in the tank, N_s is the total number of fish sampled at the beginning of an experiment and N_0 is the initial number of fish stocked.

Data analysis

The time series from the environmental surveys were analyzed to determine average daily minimum, maximun and mean values for each parameter. Pearson correlation coefficients were calculated for each time series between observations within the same series at increasing lag intervals over a period of 2 days. Magnitude and direction of the autocorrelation changes as the series is shifted down and the data points being compared drift out of phase and back into phase. This generates a wave-like pattern in which the autocorrelation coefficients oscillate with a periodicity equal to the period of the fluctuation in the time series.

For oscillating DO treatments, the total time spent under hypoxic conditions was calculated. Jobling (1994) reported limiting oxygen concentrations (LOC) in the range of 50-70% of air saturation for fish. At optimal temperature (28 °C)and unrestricted food, juvenile red drum start experiencing LOC at 5.0 mg $O_2 l^{-1}$ (73% air saturation) (Neill *et al.* 1990, 2004). LOC declines with decreasing temperatures due to the reduced metabolic activity at lower temperatures. At 18 °C Neill *et al.* (2004) estimated LOC in the vicinity

of 2.0 mg $O_2 l^{-1}$ (43% air saturation). Miller *et al.* (2002) reported a DO median lethal dose of 1.8 mg $O_2 l^{-1}$. A temperature-dependent LOC was adopted in this study

$LOC = 2.0 \text{ mg } O_2 l^{-1}$ if $T \leq 18$ °C

$LOC = -3.4 + (0.3 \times T)$ if $18 < T < 28$ °C

$LOC = 5.0 \text{ mg } O_2 l^{-1}$ if $T \geq 28$ °C

Hypoxia was defined as those DO concentrations below the computed LOC. Salinity and feeding were not factored in these calculations.

Two-way analysis of variance (ANOVA) was used to test for differences in growth and survival between treatments. In the cold front experiments repeated-measures ANOVA was employed in the analysis. Treatment and experimental time were used as sources of variation. The interaction term was used to identify treatment differences. If a main effect was detected, the analysis was followed with Tukey's HSD multiple-comparisons to test for differences between treatment means. In trials where fish were sampled only twice (beginning and end of the experiment), t-tests were used to examine differences in growth rate and survival between the different treatments. An arcsine transformation was applied to percent survival values before the analysis. All statistical analyses were computed using Systat v10.0 software, (SPSS Inc., Chicago, IL) and evaluated at an of 0.05.

RESULTS

Environmental surveys

Characterization of environmental patterns

In both seagrass beds studied, there were similar patterns of environmental fluctuations (Fig. 2.4) Seagrasses showed well-defined cycles in temperature (amplitude:

3-7 ºC), dissolved oxygen (DO) (range: 2.0-11.2 mg O_2 l^{-1}) and pH (range: 8.4-7.9) (Fig. 2.5). DO levels were high during daytime and decreased during the night to levels occasionally as low as 2.0 mg O_2 l^{-1}. The lowest temperatures and DO levels were generally reached at dawn. The percent of the day during which conditions were hypoxic varied from 0 to 38.7% (mean 23.8%). For all three time-series, autocorrelation analysis showed a clear diurnal period of fluctuation as exemplified by DO in Fig. 2.6. Daily water depth, turbidity and salinity fluctuations correlated with low-amplitude mixed tides that were characteristic of the Aransas Estuary, resulting in a time series that oscillated from 12.5 to 25 h (a diurnal phase is shown in Fig. 2.6).

Tides were the most prominent periodic feature at the INLET station. Relative to the patterns observed at SG1 and SG2, the amplitude of temperature cycles was greatly reduced at the INLET station with a daily fluctuation range of 1.0 to 1.5 ºC (Fig. 2.4). Diel DO and pH cycles were absent in the inlet, with DO levels remaining at saturation levels at all times during the study.

Spatial and temporal environmental variability

The observed daily fluctuations and effect of weather disturbances on monitored environmental parameters were almost identical at the two core areas of SG1 and SG2 (Fig. 2.7). In addition, the data obtained at the shallowest edge of the meadow at SG1 (i.e. the marsh edge) was comparable to that recorded at the core sites. In contrast, there were consistently less fluctuations in the values of the environmental parameters measured at the deep-edge location (Fig. 2.7).

Sporadic episodes during which temperature decreased more than 10 ºC within 1 or 2 days were recorded on several occasions (Fig. 2.8). These drops in temperature were associated with the passage of winter atmospheric frontal systems (fronts). Shallower areas often exhibited more drastic decreases in temperature than deep-edge locations

Figure 2.4. Temperature (top) and DO (bottom) records for seagrass SG1 (black) and SG2 (gray) stations, and INLET (dashed black) stations for a sample period of 6 days in 2000. Arrows indicates the onset of a cold front. Horizontal black and white bars represent day and night respectively.

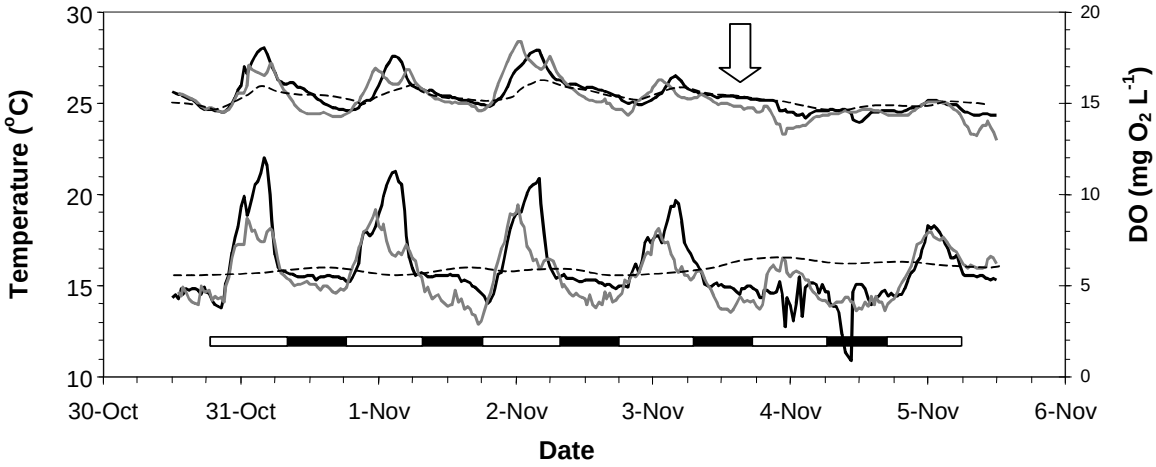


Figure 2.5. Sample time series showing daily environmental fluctuation for a period of 12 days in 2000 at seagrass bed SG2. (A) temperature (solid black), pH (solid grey), and DO (dotted black), (B) water depth (black), and salinity (grey), and (C) water depth (black), and turbidity (gray). Arrows indicate the onset of a cold front.

Figure 2.5.

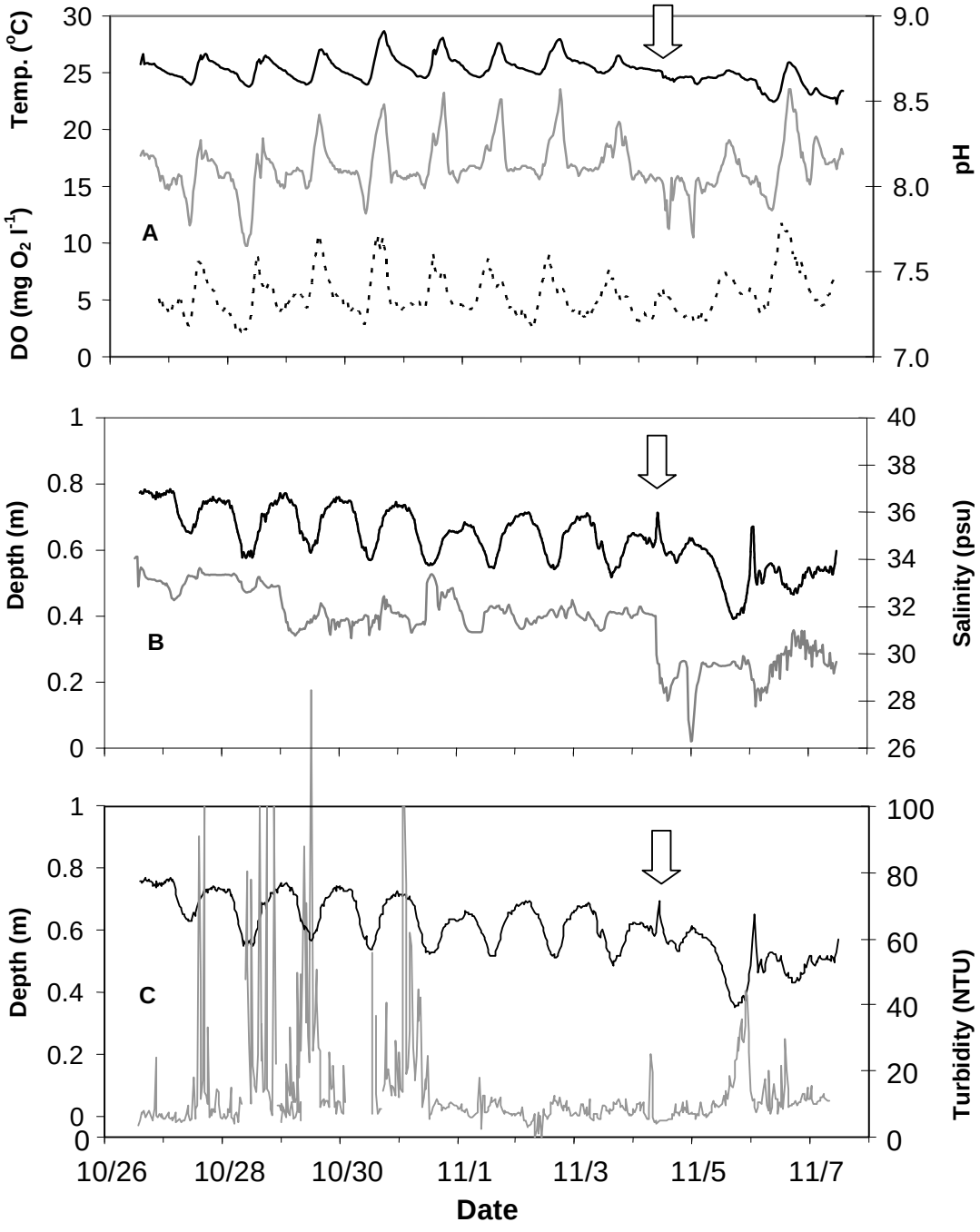

Temp. (°C)
DO (mg O2 l-1)
pH
A
Depth (m)
Salinity (psu)
B
Depth (m)
Turbidity (NTU)
C
10/26
10/28
10/30
11/1
11/3
11/5
11/7
Date

Figure 2.6. Autocorrelation plot for DO (soild line), and depth (dotted line) time series for the two first days of the data set shown in Fig. 2.4. The tide phase during the survey was diurnal.

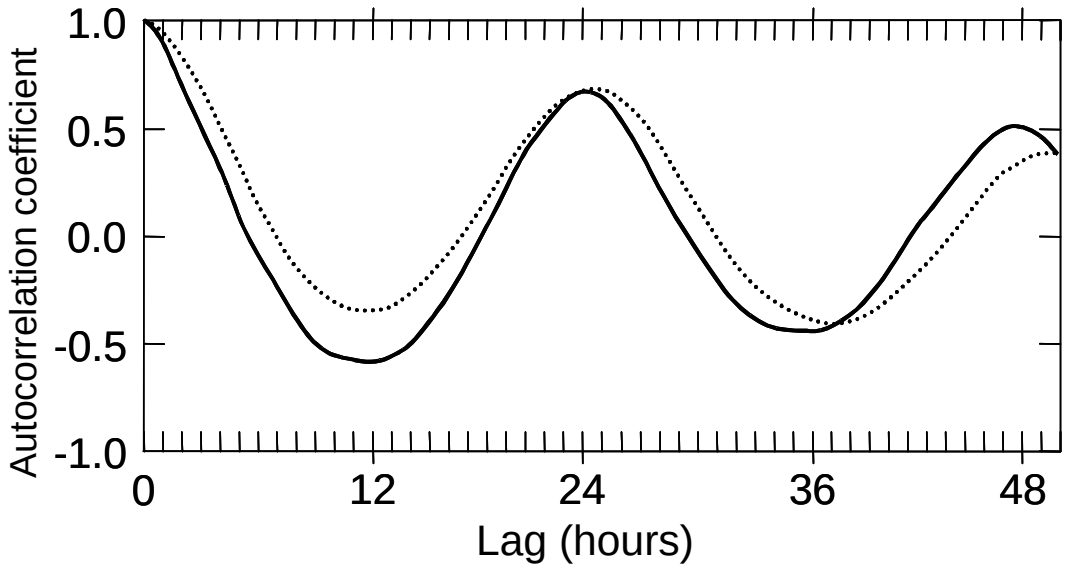
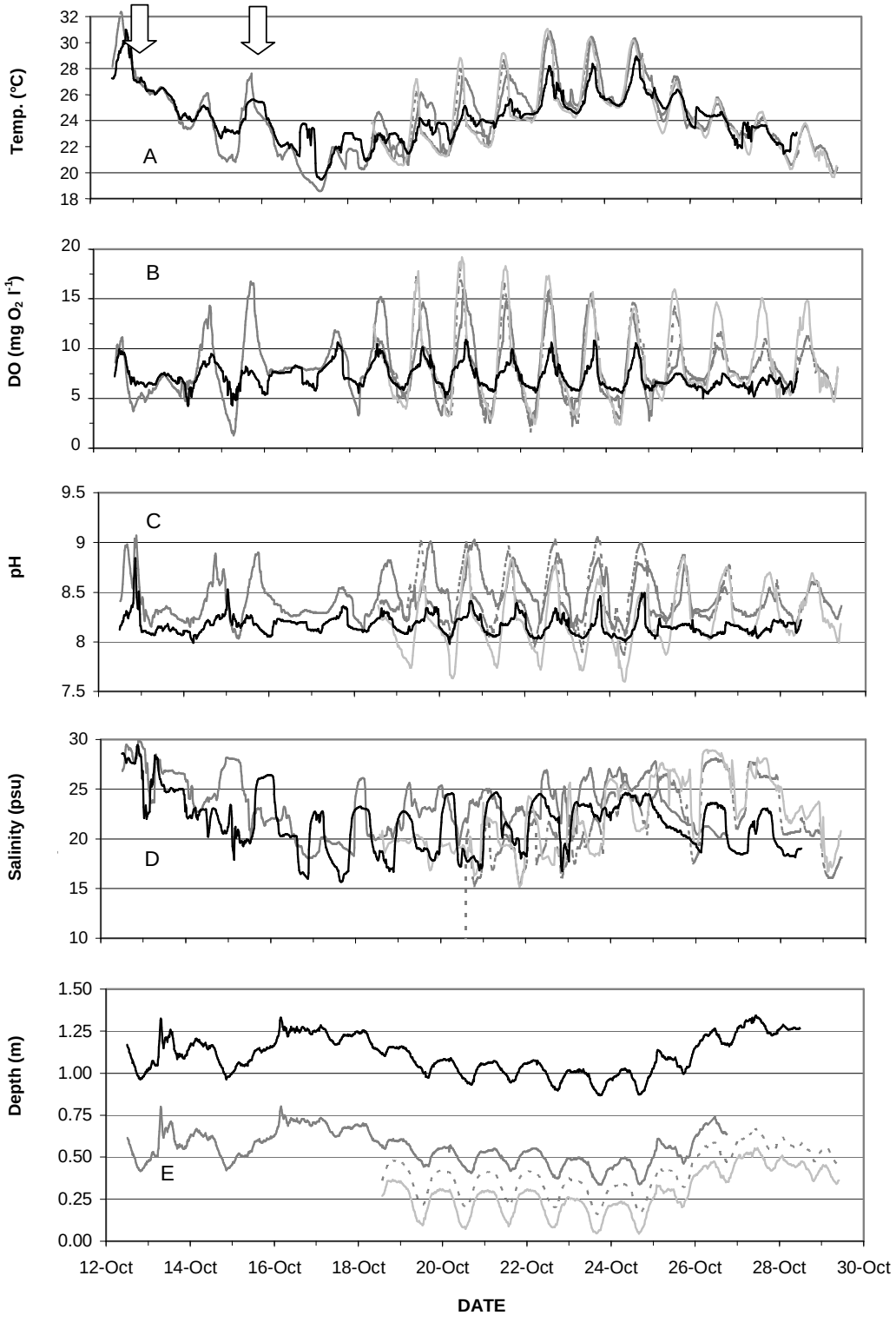


Figure 2.7. Spatial variability in environmental variables at the deep-edge (black), core1 (grey), core2 (dotted grey), and marsh edge (light grey) sites within SG1 station in 2001. (A) Temperature, (B) DO, (C) pH, (D) salinity, and (E) water depth. Arrows indicate the onset of cold fronts.

Figure 2.7.


Temp. (°C)
32
30
28
26
24
22
20
18
A
DO (mg O2 l-1)
20
15
10
5
0
B
pH
9.5
9
8.5
8
7.5
C
Salinity (psu)
30
25
20
15
10
D
Depth (m)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
E
12-Oct
14-Oct
16-Oct
18-Oct
20-Oct
22-Oct
24-Oct
26-Oct
28-Oct
30-Oct
DATE

(Fig. 2.8). Storm-related disturbances also resulted in irregularities in the tidal fluctuation and major salinity changes. In 2002, two strong tropical storms caused intense runoff that reduced the salinity to 6-8 psu (Fig. 2.8). Strong fronts were more common toward the end of the settlement season (late October and November; Fig 2.8). Weaker fronts resulted in more gradual cooling of the water but also disrupted the diel patterns of DO, pH and temperature (examples in Figs. 2.4, 2.5, 2.7 and 2.8).

Hypoxic conditions were greatly reduced or absent at the deep-edge site in comparison to the shallower ones. Hypoxic conditions ranged from 0 to 58.3% (mean 26.4%) of the day at the core and from 0 to 20.8% (mean 5.6%) at the deep-edge location. Episodic events of hypoxia were more likely to occur early in the season during the settlement on new fish in association with higher temperatures and salinities during periods of calm weather (Fig. 2.8, and 2.9). In a companion study the concurrent presence of red drum larvae was confirmed at all four sites.

Effects of diel temperature and DO fluctuations

No differences in growth were detected between diel cycling temperature treatments (TEMP1 and TEMP2). However, growth was significantly reduced relative to controls in cycling DO treatments with prolonged exposure (≥34.2%) to hypoxic levels (Table 2). Supersaturation of DO during the day failed to compensate for the prolonged exposure to hypoxia experienced by fish in the DO2 experiment. No differences were found in the growth rate of the CYCLES treatment and their respective controls. Although activity levels were not evaluated, red drum larvae subjected to low DO levels were visibly less active than control fish at dawn (pers. obs.). There were no significant differences in survival between experimental and control fish for all treatments (Table 2).

Figure 2.8. Water quality records for the seagrass deep-edge (black) and core (gray) sites of SG1 in 2002 and 2003. (A) water temperature (top), and DO (bottom). (B) Salinity (top), and depth (bottom). Arrows indicate the onset of cold fronts.

Figure 2.8.

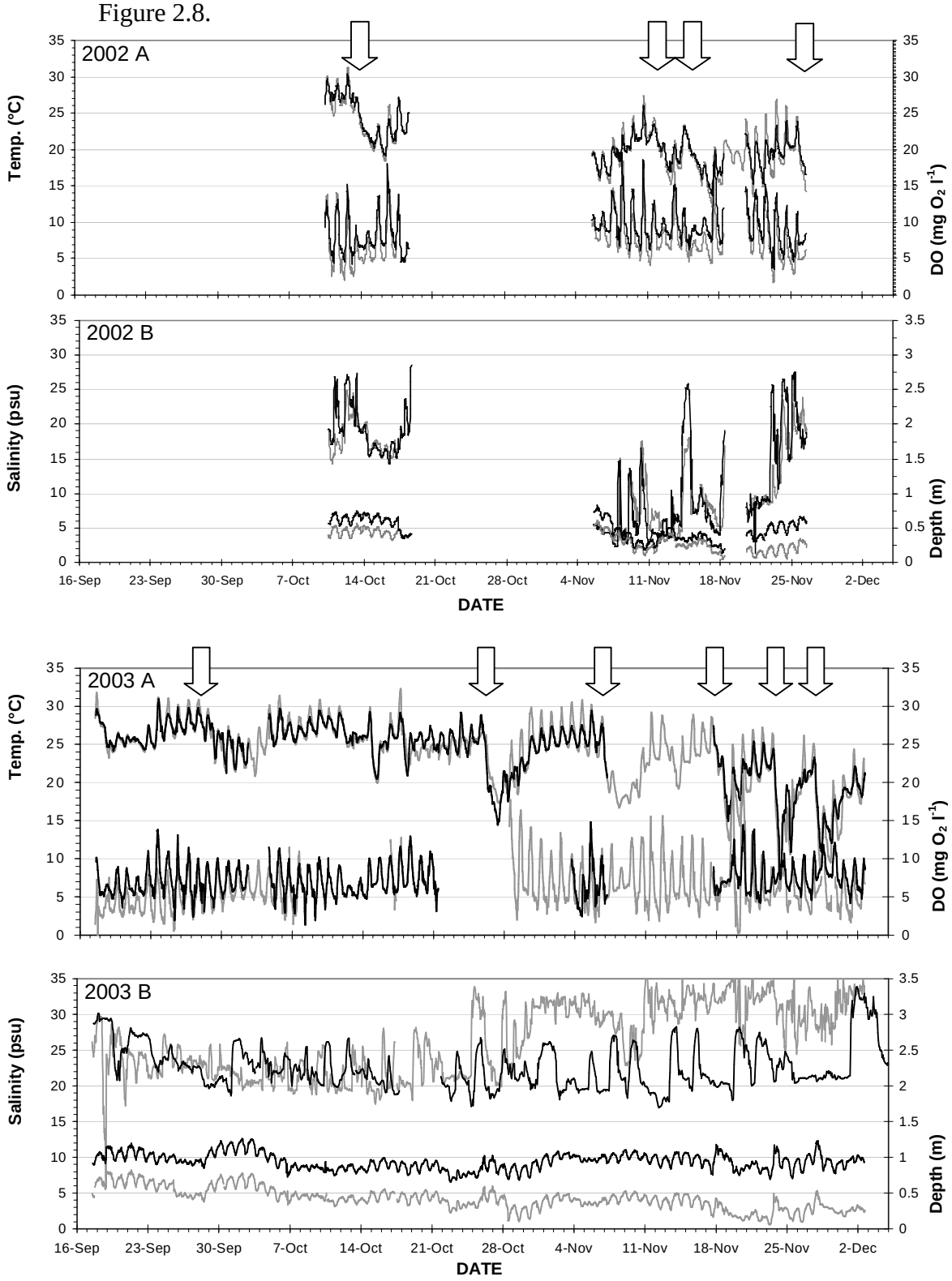

2002 A
2002 B
2003 A
2003 B
Temp. (°C)
DO (mg O2 l-1)
Salinity (psu)
Depth (m)
16-Sep
23-Sep
30-Sep
7-Oct
14-Oct
21-Oct
28-Oct
4-Nov
11-Nov
18-Nov
25-Nov
2-Dec
DATE

Figure 2.9. Relative daily duration of hypoxic events during the active settlement period and the postsettlement period in core (open bars) and edge seagrass areas (dashed bars) during 2003. Mean values + one standard deviation.

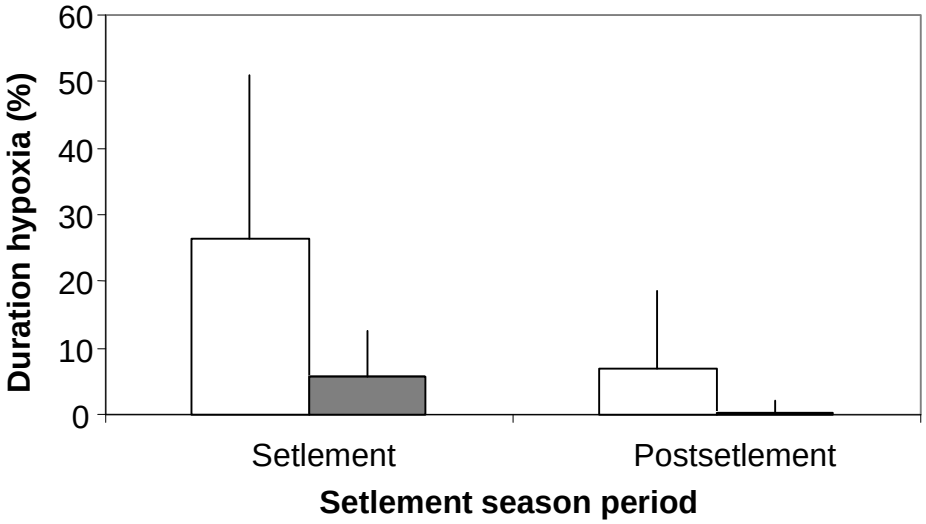


Table 2.2. Effects of various diel cycling temperature and DO treatments on the growth of red drum larvae relative to controls.

Treatment	Initial SL (mm)	Experiment duration (days)	Hypoxia duration (%)	Growth rate [1]	*P*
TEMP1	3.9	16	0	ns	0.314 [2]
TEMP2	4.9	14	0	ns	0.311 [2]
TEMP2	5.8	20	0	ns	0.931 [2]
DO1	4.6	23	57.8	reduced	0.000 [2]
DO1	4.8	13	65.5	reduced	0.036 [2]
DO2	4.6	16	34.2	reduced	0.016 [2]
DO3	4.4	22	28.7	ns	0.628 [2]
CYCLES	5.8	14	8.1	ns	0.518 [3]
CYCLES	9.6	14	8.1	ns	0.471 [3]
CYCLES	11.2	11	2.0	ns	0.604 [3]
CYCLES	16.9	7	9.6	ns	0.988 [3]
CYCLES	17.3	8	26.3	ns	0.618 [3]

[1] With respect to controls; [2] Time x treatment interaction in a two-way ANOVA; [3] Main treatment effect, two-sample t-test
ns: No significant difference (P>0.05)
No effects on survival were detected (P>0.84)

Effect of storm-related cooling

There were no differences in growth rates of larvae exposed to oscillating temperature or DO and control fish during the conditioning period ($P>0.91$) (Figs. 2.10 and 2.11), however, food consumption was higher ($P<0.01$) (Figs. 2.12 and 2.13). Cooling decreased growth rate and food consumption in all groups.

There was a two- to three-fold decrease in growth rate during the cooling phase of the simulated front. Fish previously exposed to temperature cycles had significantly faster growth rates during the cooling phase of the front ($P<0.05$) than those held at a constant temperature (Fig. 2.10). However, the difference in growth rate was no longer apparent during the recovery phase in which the temperature increased to the initial level of 27 ºC. On average, food consumption was higher for fish previously grown under cycling temperature conditions than control fish *(*$P<0.01$) (Fig. 2.12).

Fish conditioned to DO cycles grew at a rate similar to control fish during the simulated cold front (Fig. 2.11), however they exhibited greater food intake ($P<0.02$) throughout the conditioning, cooling and recovery periods (Fig. 2.13). There was no mortality during these experiments.

Figure 2.10. Instantaneous growth rates of temperature-conditioned red drum during the simulated cold front experiment. Solid columns represent fish previously exposed to temperature cycles. White columns represent control fish. Means ± SE. The *P*-value for the interaction of treatment x experimental interval two-way ANOVA is given. N represents the number of replicate enclosures per treatment. The asterisk indicates a significant difference between treatments *(P* <0.05). DD_{eff} represent effective degree-days.

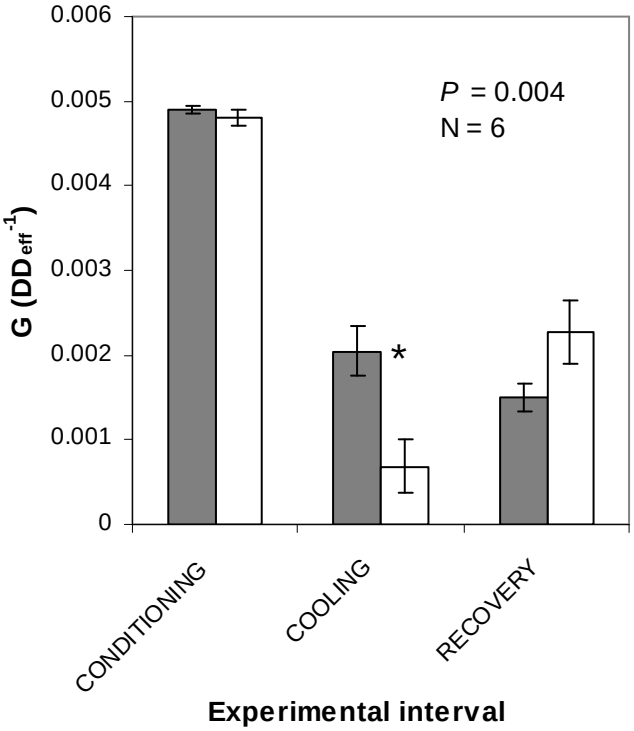


Figure 2.11. Instantaneous growth rates of DO-conditioned red drum during the simulated cold front experiment. Solid columns represent fish previously exposed to DO cycles. White columns represent control fish. Means ± SE. The *P*-value for the interaction of the treatment x experimental interval two-way ANOVA is given. N represents the number of replicate enclosures per treatment. Bars sharing the same letter are not significantly different ($P > 0.05$). DD_{eff} represent effective degree-days.

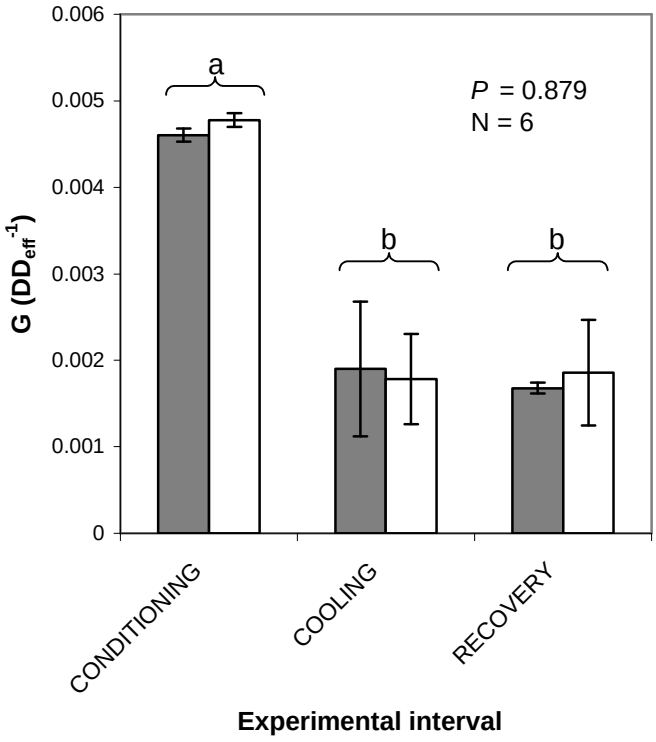


Figure 2.12. Food consumption of temperature-conditioned red drum during the simulated cold front experiment. Solid columns represent fish previously exposed to temperature cycles. White columns represent control fish. Means ± SE. The *P*-value for the interaction of the treatment x experimental interval two-way ANOVA is given. N represents the number of replicate enclosures per treatment. Bars sharing the same letter are not significantly different ($P>0.05$). BW represent body weight

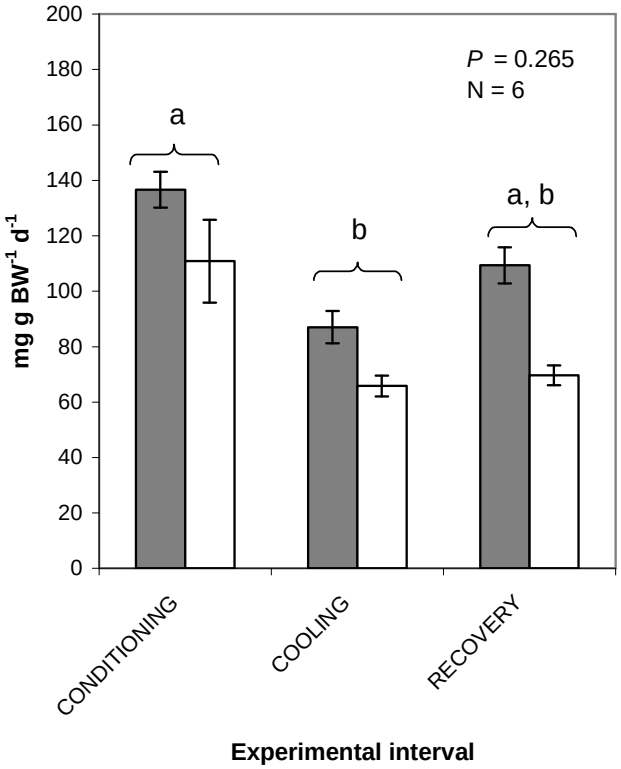
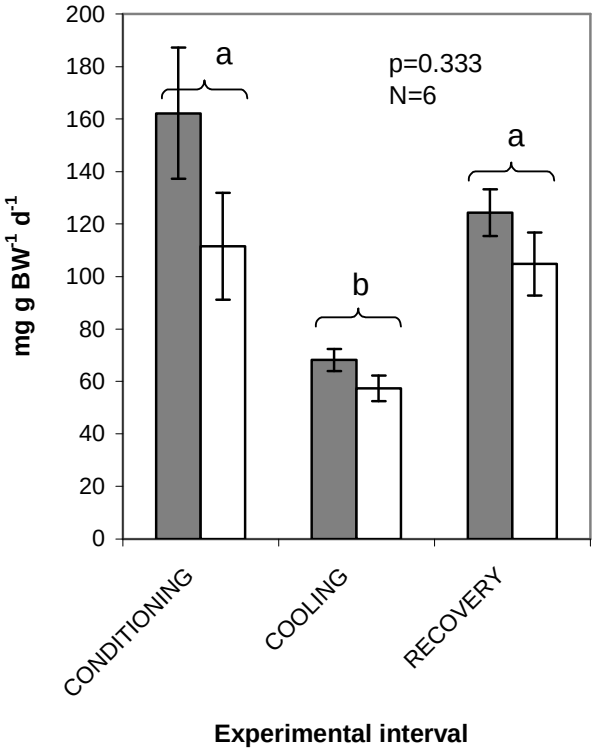


Figure 2.13. Food consumption of DO-conditioned red drum during the simulated cold front experiment. Solid columns represent fish previously exposed to DO cycles. White columns represent control fish. Means ± SE. The *P*-value for the interaction of the treatment x experimental interval two-way ANOVA is given. N represents the number of replicate enclosures per treatment. Bars sharing the same letter are not significantly different (*P*>0.05).



DISCUSSION

Naturally occurring environmental cycles are linked to different patterns of energetic input within the relatively confined nursery habitat. Circadian rhythms arise from day-night and tidal cycles, while meteorological disturbances represent stochastic fluctuations. In shallow, productive seagrass beds there is extensive primary production (photosynthesis) (Moncreiff *et al.* 1992; Ziegler and Benner 1998) and respiration confined to a small volume of water, which can result in large variations in DO concentrations during the course of a 24-h period. Temperature and DO cycles were clearly related to irradiation. Diel cycles of pH were small but very consistent and strongly correlated with DO cycles, confirming the important role of photosynthesis and respiration in the observed diel DO fluctuation. Fronts usually have a great impact on the normal diel fluctuation since they not only lower water temperature but also affect DO. In this study, diel temperature and DO cycles were weakened or disappeared during the passage of winter fronts. Strong fronts usually have major impacts on salinity and water level that result in the rapid onset of major water quality changes. An extreme example occurred in the latter half of 2002 when two storms lowered salinity to 6-8 psu. Similarly, late in 2003 three consecutive cold fronts caused sudden drops in temperature of about 15 °C in less than 24 hours. These events may result in reduced growth, and in some extreme circumstances mortality (Breitburg *et al.* 1994b; Houde 2002).

Seagrass meadows in this study were composed of two distinct habitats, the deep-edge and the shallow core zone. Deep-edge habitats are substantially more stable than core zones with respect to diel and meteorological forcing. These two zones represent the environmental conditions to which red drum larvae might be exposed following settlement. Although there is little information regarding the fine-scale distribution of

newly settled red drum larvae, the deeper edge within a meadow may provide the first adequate nursery habitat encountered by individuals, since seagrass edge is the first logical contact of new settlers with the nursery. Larvae in the process of leaving the relatively stable pelagic environment may use deep edges of seagrass meadows as transitional habitat before moving to more environmentally challenging areas (see chapter 4).

Evaluation of the effects of nursery environment conditions on wild red drum larvae depends on the assumption that the fish remain within the seagrass and are consequently exposed to the nursery conditions during the entire settlement phase. This assumption is supported by the following evidence: 1) field collections in the Aransas Estuary indicate that red drum larvae settle and stay within seagrass meadows (Rooker *et al.* 1999, Herzka *et al.* 2002), 2) red drum larvae do not undertake diel movements to and from the core seagrass area (see chapter 4), and 3) in laboratory studies, red drum larvae have affinity for and suffer less predation in structured habitat areas than open bottom (Rooker *et al.* 1998a; Stunz and Minello 2001).

Settlement-sized red drum larvae cultured in the laboratory grew at a wide range of temperatures (10-34 ºC, Fig. 2.3); growth rates increased linearly with temperature between 11 and 30 ºC. The temperature range observed during the environmental surveys performed in this study (24-32 ºC) should therefore be conducive to optimal or near optimal growth rates of settled individuals. Moreover, the simulated front experiments suggest that exposure to diel temperature rhythms may impart some advantage during rapid cooling events as compared to fish exposed to more constant environmental conditions. The simulation of the passage of frontal systems resulted in a predictable decrease in growth rate in all groups evaluated (control and cycles-conditioned) during the cooling phase. However, the growth rate reduction was significantly less for fish

previously exposed to temperature cycles. The lack of similar response in fish conditioned to DO cycles indicates that this response is probably related with the diel exposure to transient lower temperatures during the conditioning, and so can be explained as thermal acclimation response (Blaxter 1992). All groups, however, showed a similar trend in growth rate during the recovery phase, suggesting that the beneficial effect of diel cycles is short lived which further supports the acclimation hypothesis.

Fish exposed to environmentally realistic diel cycles ate, on average, more food than control fish, although they maintained similar overall growth rates. It can be inferred that the cost of growth (i.e. energy used per unit weight gain) was higher in the cycling environment. Using RNA:DNA ratios to assess nutritional condition, Rooker *et al*. (1997) concluded that red drum in seagrass beds were well-fed and starvation was rare. Herzka and Holt (2000) found that the isotopic composition of settlement-size red drum changed significantly faster in larvae stocked in cages in the field than siblings held in the laboratory and concluded that metabolic turnover (changes in isotopic composition not explained by growth) was accelerated in the caged fish. Faster metabolic turnover is likely to indicate the joint effects of increased respiration (i.e. metabolic work not related to growth) and protein turnover (Hawkins 1991; Joblin 1994; Houlihan *et al.* 1995) under the environmental conditions in the nursery. Similarly, fish exposed in the laboratory to diel cycles may have higher fixed metabolic costs or increased activity costs in response to the changing environment, which are expected to narrow the metabolic scope for growth (Fry 1947, 1971; Wieser and Medgyesy 2000) that may result in reduced growth. However, the benefit of evolving a nursery-dependent lifestyle strongly argues for specific tradeoffs and adaptations to meet the environmental challenges of these fluctuating environments. The observed increase in food consumption in the laboratory may be a compensatory response to offset the challenges imposed by environmental

fluctuation characteristic of nursery grounds. In any case these observations call for more detailed research of growth mechanisms under daily fluctuating environmental conditions.

Finally growth rates during the recovery phase remained low while food intake increased to levels similar to those of the conditioning phase. Hyperphagia is usually involved in the compensatory growth response (CG) after a period of growth depression due to reduced food supply or temperature (Ali *et al.* 2003; Nicieza and Metcalfe1997). Results suggest a lag in the onset of the recovery process for growth compared to appetite, and that the recovery phase was not sufficiently long to detect changes in growth rates. Nicieza and Metcalfe (1997) reported a similar time lag for the onset of CG for juvenile Atlantic salmon (*Salmo salar*) when growth was restricted for a period of time by low temperatures.

For juvenile red drum, DO starts to be limiting at about 5.0 mg O_2 l^{-1} at 28 ºC (LOC approx. 74% air saturation) (Neill et al. 1990). However, limiting DO sharply declines to about 2.0- 3.0 mg O_2 l^{-1} at 18- 21 ºC (Neill et al. 2004). All laboratory DO treatments repeatedly exposed red drum larvae to oxygen tensions below the limiting thresholds (i.e. hypoxia). However, only the treatments in which larvae were subjected to severe limitation of dissolved oxygen (DO1 and DO2) resulted in lower growth rates relative to control fish. Juvenile southern flounder (Paralichthys lethostigma) experience blood chemistry changes while exposed to episodic hypoxia, however they must remain in moderate but continuous hypoxia to acclimate to low DO (Taylor and Miller 2001). It is worthy to note that diurnal hyperoxia did not make up for the nocturnal hypoxia (DO2) in terms of growth rates. In fish, hyperoxia causes transient acid-base imbalances (Wood and LeMoigne 1991) as the fish hypoventilate. However, there is no evidence in the literature that DO levels above saturation result in significant growth impairment

(Caldwell and Hindshaw, 1994; Person-Le Ruyet et al. 2002; Foss et al. 2003). Ectotherms have been proposed as 'multi-stable' systems in contrast to the homeostatic paradigm of endothermic organisms (see discussion in Duthie and Houlihan 1982). Fish may function reasonably well in a range of environmental conditions and avoid costly acclimation responses within certain tolerance limits dictated by the evolutionary history of the species. It would be very interesting to determine the hourly energy budgeting in environments with important diel components and how available energy is translated into growth.

The cumulative amount of time that fish were exposed to hypoxia in the laboratory was probably the cause of growth reduction in the laboratory experiments. The total time spent in hypoxic conditions was used to measure the severity of hypoxia experienced by the fish. Laboratory results suggest that growth is impaired when hypoxic events last for more than 8.2 h d^{-1}. Consequently, this value could be a criterion that defines poor nursery habitat quality. In the relatively pristine seagrass beds studied, hypoxia is reached repeatedly, especially at the beginning of the settlement season when temperatures and salinities are relatively high and the weather is relatively stable. However, hypoxic conditions typically last for less than 6.3 h d^{-1} in the core of seagrass meadows and less than 1.3 h d^{-1} in the deepest edge. Consequently, short hypoxic events might not limit the overall growth performance of larval red drum in the nursery habitat. On the other hand, eutrophication from anthropogenic impacts on seagrasses may increase production (Bostroem *et al.* 2002; Deegan, 2002). Ziegler and Benner (1998), working in pristine seagrass, reported that diel DO changes were linked to local benthic processes. The strong dependency of DO oscillations on day-night cycles and weather events found in my surveys indicates that DO cycles are essentially driven by local productivity. Consequently, eutrophication could result in prolonged periods of hypoxia.

Beck and Bruland (2000) reported diel DO fluctuations varying from >250% saturation to complete anoxia under euthropic conditions in a shallow tidal salt marsh. This may represent an extreme scenario. However, depending upon the severity of the disturbance, environmental conditions within seagrasses could degrade quickly, thereby increasing the amplitude of DO fluctuation and hypoxia events. These conditions could affect red drum growth and therefore the value of the seagrass as nursery habitat.

CONCLUSIONS

This study indicates that field estimates of environmental characteristics based solely upon once daily measurements is inadequate for predicting fish growth in habitats characterized by marked environmental heterogeneity. For example, since oscillating environmental conditions with severe but transient hypoxia would reduce growth in red drum larvae, then a single daily DO measurement made during daytime would be insufficient to capture growth-retarding hypoxic periods and growth potential for the habitat would be overestimated. Environmentally realistic temperature and DO cycles neither improved nor impaired red drum growth but seemed to offer some advantage in dealing with extreme environmental changes, as suggested in the cold front experiments. At settlement, red drum encounter a new and very dynamic habitat where rapid diel environmental changes are probably too short to induce an immediate acclimation response to any particular combination of environmental factors. However they may allow for general physiological changes that acclimate their metabolism to a range of conditions. Since both past experience and present conditions can be important, performance estimates under constant conditions have to be used with caution in predicting responses in fluctuating environments. Larval growth and survival in fluctuating environments of estuaries is a crucial aspect that may help to explain some aspects of recruitment variability in estuarine dependent species like red drum.

Chapter 3: Changes in whole body cortisol and thyroid hormone levels during red drum (*Sciaenops ocellatus*) larval development; effects of nursery environmental cycles on growth, survival and cortisol content during settlement

ABSTRACT

Red drum settle into shallow seagrass meadows during the larval stage. Day-night cycles in these habitats result in marked diel temperature and dissolved oxygen (DO) fluctuations that may result in stress and reduced growth. This study detected an early activation of thyroid and interrenal glands during the yolk-sac phase and a second activation of the thyroid gland during transformation into juveniles. Whole-body L-thyroxine (T4) and cortisol concentrations showed large diurnal rhythms (2-12 ng g^{-1}, and 0.5-2.5 ng g^{-1} respectively) during settlement, whereas diel fluctuations in whole body 3-5-3'-triiodo-L-thyronine (T3) levels were detected in early settlers (7.9 mm SL) but not in older individuals. All hormones showed a decline during the night. Settlement-size larvae exposed to a strong environmental stimulus increased whole body cortisol. Additionally, settlement-size larvae exposed to various laboratory-generated temperature or DO cycles showed no difference in growth compared to fish grown under stable conditions (control). However, growth rate was significantly reduced in DO cycled fish with prolonged exposure to hypoxia. No differences were found in whole-body cortisol levels in the reduced growth treatment groups, suggesting that growth retardation was not related to a cortisol-mediated stress response. In moderate DO and temperature treatment groups, cortisol showed wider fluctuations than control groups during the night time that were not related to stress. I conclude that: 1) red drum activated T3 and cortisol production during the yolk-sac phase; 2) full functionality of the interrenal and thyroid glands is probably achieved around settlement stage; 3) environmentally realistic

temperature and DO cycles do not result in a cortisol-mediated stress response by larvae; and 4) fish growth was reduced with transient but severe hypoxia, however, no cortisol stress response was elicited.

INTRODUCTION

Reproduction in red drum, as in most marine fishes, results in large numbers of poorly developed pelagic larvae released into the environment (Winemiller and Rose 1993; Wilson and Nieland 1994). During the larval period, fish undergo a suite of complex morphological changes (ontogeny) and a remarkable size progression (growth) (Fuiman 1998, 2002; Fukuhara 1991) that takes them from a free-swimming embryo to a competent juvenile in a very short time span. Development and growth rates are much higher in the larval phase than in any other phase of the life cycle (Blaxter 1986: Fuiman 2002), as is mortality. Losses for most marine teleosts exceed 99% during the larval period (Houde, 2002). High mortality is partially attributable to the incomplete state of development of key organs (i.e. digestive, osmoregulatory, sensory, or endocrine) that are essential for survival (Tanaka *et al.* 1995; Infante and Cahu 2001). Growth and ontogeny, and the addition of new or improved physiological competence follow a well-defined and genetically-programmed sequence in which hormone regulation is critical (Brown and Bern 1989). Ontogenetic hormonal surges are related to pre-adaptive changes in fish physiology (Specker 1988; Tanaka *et al.*1995) and prepare the organism to deal with habitat transitions such as emergence in chum salmon (*Oncorhynchus keta*) (Tagawa and Hirano 1990; Iwata *et al.* 2003), settlement in flounder (*Paralichthys olivaceous*) (de Jesus *et al.* 1991) and black sea bream (*Acanthopagrus schlegeli*) (Tanaka *et al.* 1991), freshwater migration in Japanese seabass (*Lateolabrax japonicus*) (Perez *et al.* 1999), and smoltification (Specker 1988). The small size of teleosts larvae requires the use of

extraction procedures and the pooling of tissue from a few to hundreds of larvae. Due to these limitations, most studies have focused on the development of the thyroid system (thyroid hormones) and the interrenal gland (cortisol) for which suitable extraction techniques are available (Tanaka 1995). Across vertebrates, thyroid hormones are implicated in growth and development (Brown and Bern. 1989; Inui *et al.* 1995; Oppenheimer and Schwartz 1997), and metabolic rate regulation (Power *et al.* 2001; Goglia *et al.* 2002). Cortisol is the single product of the interrenal gland in teleosts, and has both gluco- and mineralocorticoid actions. Cortisol has direct effects on development (Sangild *et al.* 1994), metabolism (Pickering and Pottinger 1983; Monnsen *et al.* 1999; Hart and Pitcher 1999), immune system (Bateman *et al.*1989), and stress (Wendelaar Bonga 1997).

Many marine fishes use distinct nursery habitats during early life. These habitats provide shelter (Rooker *et al.* 1998a) and sufficient food to sustain high growth rates. Red drum settle into shallow seagrass meadows and marsh edges during the larval stage at sizes ranging from 5 to10 mm standard length (SL) with peak settlement at 6-8 mm SL (Holt *et al.* 1983, Rooker 1998; Herzka *et al.* 2002, Stunz and Minello 2001). Day/night cycles in these nursery habitats result in marked diel temperature cycles and dramatic changes in photosynthesis:respiration ratios leading to large dissolved oxygen (DO) fluctuations that may cause transient hypoxia at dawn (chapter 2). Extreme environments may cause stress and reduced growth (Van Weerd and Komen, 1998; Andersen *et al.* 1991). On the other hand, these same environmental cycles may stimulate animals to initiate directed changes in activity, physiology and behavior (Fry 1947 and 1971; Pihl *et al.* 1991; Diaz *et al.* 1995) that result in compensatory or anticipatory metabolic responses and avoidance behaviors (Fontaine 1993, Breitburg 1994).

In addition to ontogenetic changes in hormone content, fishes often have daily rhythms of circulating hormone levels that respond to both endogenous and environmental cues (Leatherland 1982; Pickering and Pottinger 1983; Meier 1992; Leiner and MacKenzie 2003). Most studies show that fish activity indeed follows a circadian rhythm, suggesting that light/dark cycles are the main oscillator which is fine-tuned by numerous directive factors in the environmental (i.e. temperature, tides, lunar phase, food abundance, etc., Gerkema 1992). Holt and Holt (2000) documented a clear diel pattern in gut fullness and vertical distribution in presettlement red drum larvae. Similar patterns have been described for haddock (MacKenzie *et al.* 1999) and Atlantic menhaden (Forward *et al.* 1999) larvae. Available information regarding diel physiological changes in larval fish is entirely limited to biochemical indices of condition. In field-caught larval red drum, RNA:DNA ratios oscillated during the day in close agreement with ambient temperature (Rooker *et al*. 1997) although diel RNA:DNA ratios were not temperature dependent (Rooker and Holt 1996) in the laboratory. Chymotrypsin activity levels in red fish larvae increase significantly during the light phase, probably reflecting feeding (Applebaum and Holt 2003). These patterns of activity suggest a precise temporal order of physiological and behavioral responses to environmental factors that are probably under hormonal control (Denver 1997). To my knowledge no information is available on the developmental and diel profiles of hormone production in larval red drum.

In fish, environmental stimuli above tolerance thresholds, such as acute drops in temperature, physical disturbances, hypoxia, and pollutants, can elicit increases in cortisol and catecholamine in plasma and tissue (Wendelaar Bonga 1997). This primary stress response, is considered adaptive in allowing fish to deal with the stressor and maintain homeostasis by regaining control of physiological processes or by movements away from the stressful environment. This response is transient and in most cases cortisol

decreases to or slightly below pre-stress levels within a few hours after removal of the stressor (Robertson et al. 1988; Barry *et al.* 1995, Ackerman *et al.* 2000). If stressors are chronic or too severe the stress response may exceed physiological tolerances and become maladaptive leading to growth retardation, poor food conversion and weight loss, or depression of the immune system. Information on the development of the cortisol stress response during early ontogeny of marine fish larvae is completely lacking.

The goals of this paper are to: 1) describe the first appearance of the thyroid and interrenal glands and document changes in hormone levels during the larval stage up to final metamorphosis into juveniles with special emphasis on settlement sizes; 2) determine diel patterns of thyroid hormones (TH) and cortisol production in settlement-size larvae; 3) determine the responsiveness of red drum larvae to a host of environmental stimuli; and 4) determine the effects of laboratory-generated temperature or DO cycles on larval growth, survival, and cortisol production, and its possible role as mediator of directive environmental factors.

MATERIAL AND METHODS

Endocrine development

Ontogeny of TH and cortisol production

Fertilized eggs of red drum were obtained from several broodstock induced to spawn naturally under temperature and photoperiod control at the Fisheries and Mariculture Laboratory, Marine Science Institute (Port Aransas, Texas). Spawning occurred shortly after dusk. Eggs were collected the following morning and examined to confirm fertilization and record the developmental stage of the embryo. At least three pools of eggs (ca. 200 mg wet weight) were frozen (-80 ºC) for hormone measurements.

About 30,000 eggs were stocked in two 350-l cylindrical rearing tanks supplied with a constant flow of seawater (25 ml min^{-1}). Temperature was kept constant at 27 °C, and salinity ranged from 26 to 32 psu and initially matched that of the spawning tank. Larvae hatched 21- to 24-h after spawning. Hatching rate was estimated from the average number of live larvae in five 100-ml water samples; only batches with a hatching rate greater than 95% were used for the experiments. Starting 2-3 days post-hatching (dph) until 10 dph, rotifers (5 ml^{-1}) enriched in live cultures of *Isochrysis galbana* (UTEX LB 2307) were offered twice daily. Additionally, a particulate larval dry feed (Kyowa B 250µm; Kyowa Hakko Kogyo Co., Ltd. Tokyo, Japan) was continuously added during the daytime to the tank through automatic feeders. After day 10, live food was withdrawn and only dry feed of increasing particle size was used through the end of the rearing at 40 dph. Photoperiod was 12L:12D (light:dark). All rearing protocols and experimental methods followed approved animal care directives.

Samples were taken daily from 1 to 5 dph, and at increasing time intervals thereafter (see figure 3.3). Sampling was done within one hour of the start of the light phase and before fish were fed. For whole-body hormone content, a pool of young larvae or 6-9 late-larvae/juveniles, each weighing more than 150 mg (wet weight) were sampled from each rearing tank. Fish were quickly netted out of the tanks, restrained on ice for few seconds, blotted dry on filter paper and immediately frozen on dry ice and stored at –80 °C for hormone extraction. The whole sampling procedure was usually done in less than 1 min. An additional sample was taken for morphometric and histological observations. Fish were sacrificed with a lethal dose of tricaine methanesulfonate (MS222) and either preserved in 5% buffered formalin (35-60 individuals, for morphometry), or fixed in Bouin's solution for 24 hours and then preserved in 70% ethanol (10-15 individuals, for histology). Development of thyroid and interrenal glands

was examined on histological sections of at least two individuals at different ages throughout the larval and early juvenile period. Briefly, fish were dehydrated through a series or graded alcohol and xylene solutions and finally embedded in paraffin. Sections were later cut at 5 um thickness and stained with haematoxylin and eosin (HE). Identification of thyroid and interrenal glands was done according to Takashima and Hibiya (1995). Since developmental progress through the larval period in larval fishes is closely related to SL (Fuiman *et al.* 1998), size was used to estimate the developmental stage of the fish. Newly hatched to 5 mm SL larvae were measured under a dissecting microscope fitted with a camera lucida. This system superimposed on the image of the larvae a digitizing tablet (Summa Sketch II) connected to a computer. Standard length (SL) was traced on the tablet using SigmaScan Pro 4.01.003 software (SPSS Inc., Chicago, IL). Larvae larger than 5 mm and juveniles, were photographed with a reference scale (used for calibration) and later measured using ImageJ 1.30v software (http://rsb.info.nih.gov/ij/). Standard length was measured to the nearest 0.01 mm.

Diel profiles of TH and cortisol production

Diel profiles of hormone production were monitored at two ontogenetic stages, 4.5 mm SL (19 dph) and 14.5 mm SL (30 dph) that correspond to early settlers and post-settlement larvae respectively. The early larvae were sampled alternatively from each one of the two 300-l rearing tanks. Samples consisting of three pools of 150 mg wet weight were taken every 3 h for 36 hours (12 samples). Night time samples were collected with the help of a red light. Care was taken not to disturb the remaining fish in the tank during sampling. A different experimental design was used for diel experiments on late-larvae. Groups of 60-80 fish were randomly transferred to 18 flat-bottom tanks (75-l) connected into a common recirculation system. After subjection to the new environment (36-48 h) the tanks were sampled one at a time every 3 h as described before. This design made the

sampling more efficient for fish with improved swimming abilities and avoided stress increase associated with repetitive sampling from the same tank. Fish were fed manually four times per day.

Cortisol response to environmental stimuli

Handling

Approximately 35 red drum larvae were randomly transferred to ten circular enclosures (0.35 x 0.45 m, 750 µm bottom mesh) placed within ten 125-l tanks. After the acclimation period (24-36 h), one mesocosm was quickly sampled for baseline cortisol estimation. Fish in the remaining nine enclosures were subjected to an acute physical stress as follows. The enclosures were completely lifted out of the water for 1 min and then returned to the tank where strong aeration was maintained for an additional minute. Finally the aeration was turned down and the fish allowed to recover to the same conditions prior to the stress administration. This handling procedure was first tested in juvenile red drum (26.3 mm SL) and proved to elicit a strong cortisol response. One enclosure was sampled immediately after the stress administration (time 0) and the rest at different times after the stress (see figure 3.9) for a 24-h recovery period. The experiment was conducted at two different ontogenetic stages (7.9 and 9.8 mm SL) corresponding to the range of sizes that peak settlement occurs in the field (Holt *et al.* 1983, Rooker *et al.* 1998b; Herzka *et al.* 2001, Stunz *et al.* 2002). Sampling was conducted as described before and samples kept frozen (-80 °C) until hormone extraction.

Fluctuating environments

Groups of 300 red drum larvae were randomly transferred to six 120-l flat-bottom experimental aquariums arranged in two separate recirculating rearing systems and allowed to experience the new environment for 24 h. At the end of this period larvae had

reached 5.2-6.0 mm SL, sizes that correspond, with initial settlement of wild red drum larvae. After the exposure period (acclimation), three simulated diel environmental cycles (TEMP, DO_{mod} and DO_{sev}, described below), were generated in one of the recirculating systems, while the other system served as control (no cycles). All treatments were tested independently and performance was compared to siblings kept at uniform temperatures (27 ± 0.2 °C) in constantly well-oxygenated water (6.1 ± 0.7 mg O_2 l^{-1}, Control). The cycling temperature treatment (TEMP) was chosen to reflect high amplitude diel cycles (27 ± 3.0 °C), characteristic of the nursery habitat. The two diel DO cycles were designed to result in two distinct levels of exposure to nocturnal hypoxia. The hypoxia threshold for red drum larvae was based on estimates done for juvenile red drum and set at 5.0 mg O_2 l^{-1}. DO_{mod} (3.5-12.8 mg O_2 l^{-1}) was designed to provide a moderate oxygen limitation at dawn (<1 h d^{-1} total hypoxia) reflecting environmentally realistic oxygen fluctuations based on environmental surveys of the nursery habitat (chapter 2). In contrast DO_{sev} (2.4-9.4 mg O_2 l^{-1}) resulted in a more severe oxygen limitation (~7 h d^{-1} total hypoxia) characteristic of some eutrophic estuarine systems (Robbins and Bell 2000). All tanks subjected to oscillations in DO and their respective controls were kept at constant temperature (27 ± 0.2 °C). To generate the desired fluctuating temperature and DO conditions we used timer-controlled heaters combined with a water chiller unit, and a countercurrent gas depletion column with timer-controlled solenoid valves controlling the injection of nitrogen or oxygen into the column respectively. Predictable fluctuating regimens were obtained by trial-and-error manipulations of the timer's switching times (cycle timing), and intensity of heat and gas injection (cycle amplitudes). Water was recirculated through each system 1.2 times h^{-1} to ensure homogeneous environment among the three replicate tanks within a treatment. Temperature, DO, pH and salinity were recorded in at least one tank from each treatment at 15- or 30-min intervals by a

multiparameter water quality data sonde (YSI Inc., Yellow Springs, OH). Approximately 10% of the total water volume was exchanged daily. Photoperiod was 12L:12D and food was dispensed constantly through automatic feeders during the light period. Experiments lasted for 10 to 16 d.

A total of 20-25 fish from each tank was sampled at regular intervals throughout each experiment (see table 3.2 for details) and kept in 5% buffered formalin and later measured. Samples were also taken for whole-body cortisol measurements on day 10 and 15 at noon. An additional nighttime sample was collected on day 15. For whole-body hormone content, one to six pools of larvae weighing more than 50 mg (wet weight) were sampled from each rearing tank totaling 3 to 18 replicate pools per sampling date and treatment combination. On the last sampling date two samples were collected, a daytime sample (noon) and a nighttime (dusk or dawn) sample, to measure possible diel physiological changes in cortisol. Statistical differences between control and treatment were analyzed using two-way analysis of variance (ANOVA) and a nested tank design was employed in the analysis. All statistical comparisons were conducted using Systat v10.0 software (SPSS Inc., Chicago, IL).

Sample extraction and hormone assays

Whole body cortisol

Larvae or groups of larvae weighing 50-200 mg were homogenized in 5-6 times volume by weight of ice-cold phosphate buffer saline, PBS (0.01M, 0.15M NaCl, pH 7.4). The homogenates were placed in an ultrasonic bath at 4 °C for ten minutes, and then a volume of homogenate containing at least 50 mg tissue was transferred to a clean tube and extracted with 4 ml ethyl ether twice (de Jesus *et al.* 1991). The ether was later evaporated under a stream of nitrogen (2 to 3 h) and the extracts resolubilized in 150 µl of

PBS-Gelatin (0.01M, 0.15M NaCl, 0.1% Gelatin, 0.001% NaN_3, pH 7.4) and defatted with carbon tetrachloride as described by Hiroi *et al.* (1997) prior to radioimmunoassay (RIA) (see appendice A). Recovery efficiency was calculated from known additions of tritiated cortisol (500, 1000 and 10,000 cpm, hot spikes) to larvae homogenates before extraction. After the extraction the samples were counted again to determine the percentage of remaining radioactivity. The average recovery was 71.4 ± 3.0% (mean ± SD, n=9). Additionally, larvae homogenates were split in two halves and one of them spiked with known amounts of unlabelled cortisol (cold spikes), and then both extracted and assayed. These tubes were included in most extractions. Percent recovery was estimated from the difference in cortisol content between spiked and unspiked homogenate. The average recovery was 71.9 ± 6.9% (mean ± SD, n=12).

Cortisol radioimmunoassay (RIA) was conducted according to previously validated assays for fish larvae homogenates (Yamano *et al.* 1991, Hiroi *et al.* 1997). The cortisol antiserum was purchased from Esoterix Inc. Endocrinology, Calabasas Hills, CA (catalog no. F3-314), and the radiolabeled cortisol from PerkinElmer, Boston, Ma (catalog no. Net-396), all other reagents were obtained from ICN Biomedicals (Aurora, OH). The sensitivity of the cortisol RIA was 0.07 ng ml^{-1} (lower limit of the 95% confidence interval of the zero binding "B0", n=12). Intraassay coefficients of variation (CVs) at mean cortisol binding level of 80.0 and 62.5% were 6.25 and 5.28% (n=50), respectively, and interassay CVs at mean binding of 50% was 9.84% (n=12). The minimum detectable cortisol dose from 50 mg tissue (80% Bi/B0 in the RIA) after factoring the extraction and assay procedures was 0.19 ng g^{-1}.

Whole body thyroid hormones triiodothyronine (T3) and thyroxine (T4)

The methodology used in this study combined a first step in which thyroid hormones were extracted from larval tissues according to standard procedures currently

applied to fish eggs and larvae, followed by two enzyme immunoassays (EIA) newly developed for quantification of T3 and T4 in the extracts. Thyroid hormones were extracted from the same homogenates as those used for cortisol, and follow the method described by Greenblatt *et al.* (1989) with some minor modifications. The homogenate was extracted twice with 6 and 2 ml ice-cold methanol. The methanol was evaporated in a rotary evaporator overnight. The dry extracts were resolubilized in 50 µl **Barbital Buffer** (0.1M, 0.1% Gelatin, pH 8.6) and 50 µl methanol and lipids were removed with 200 µl chloroform. The aqueous phase was transferred to a fresh tube, lyophilized and finally stored dry at –20 °C prior to the enzyme immunoassay (EIA) (see appendice A). Recovery efficiency was estimated from known addition of hormone standards (cold spikes) to larvae homogenates as described for cortisol. The average recovery was not statistically different between T3 and T4 (Student's t-Test, $P = 0.457$) and averaged 82.1 ± 6.9% (mean ± SD, n=6).

Two specific EIAs were developed for the quantification of T3 and T4 in tissue extracts of red drum larvae. The reagents and solutions used in the assay were: coating buffer, sodium carbonate-bicarbonate buffer (0.1M, pH 9.2); blocking buffer, PBS (0.1M, NaCl 0.15M, pH 7.4), KCl 0.003M, 0.25% Bovine Serum Albumin (BSA); washing buffer, PBS (0.01M, NaCl 0.15M, pH 7.4), Tween 20® 0.05%; EIA buffer, PBS (0.1M, NaCl 0.15M, pH 7.4), Tween 20® 0.01%, BSA 0.1%; Horseradish peroxidase (HRP) substrate solution (ImmunoPure® TMB Substrate kit, Pierce, Rockford IL, catalog no. 34021); and stopping solution, sulfuric acid (2M). Standard T3 and T4 were purchased from ICM (catalog no. 152170 and 152145, respectively). The anti-T3 antiserum was obtained from Fitzerald Industries, Concord, MA (catalog no. 10-T35, clone M94210) and the anti-T4 from Biodesign International, Saco, Maine (catalog no. E20652M, clone ME.125). Tracers, T3 HRP and T4 HRP conjugates, were purchased from OEM

concepts, Toms River, NJ (catalog no. H6-T01-2 and H6-T02-2, respectively). All other reagents were obtained from ICN Biomedicals.

The EIA format was a competitive immunoassay in which the specific antibody served as primary and capture antibody. The EIA assays were conducted in 96-well polystyrene Microtiter plates (Immulon® 4HBX, Thermo Labsystems, Franklin, MA, catalog no. 3855) as follows. Coating. The specific antiserum was diluted in coating buffer and 100 µl of the solution was added to all but the top well of the first two columns (nonspecific binding wells, NSB). The plate was sealed and incubated overnight at 4 °C. The wells were emptied after incubation, rinsed with 400 µl of water, and 150 µl of blocking buffer was added. The plates were incubated for 3 h at room temperature and then stored at 4 °C. Coated plates could be stored in this condition for at least 4 d. Plate design and components incubation. On the day of the assay the plates were rinsed three times with washing buffer and gently shook upside down over filter paper to remove any excess buffer. The top two wells of the first two columns on each plate received 100 µl of EIA buffer each (NSB and B0). The remaining 12 wells of the first two columns received 100 µl of hormone standard diluted in EIA buffer. The standards ranged from 0.08 to 20 ng ml^{-1}. The extracted samples were resuspended with 425 µl EIA buffer and 100 µl added to the plates in duplicate. The plate was then covered and preincubated for 3-4 h at room temperature. After the preincubation time 25 µl of tracer solution in EIA buffer (T3-HRP, 0.5 Eu ml^{-1} and T4-HRP, 0.3 Eu ml^{-1} final concentration) was added to each well. The plate was finally sealed and incubated overnight at 4 °C. Plate development. Plates were rinsed five times with washing buffer and gently shook over filter paper. One hundred µl of the HRP substrate solution was added to all wells, and incubated for 25 min at room temperature. The reaction was stopped with 50 µl of stopping solution and the absorbance immediately measured at 450 nm with background wavelength correction set

at 620 nm (Spectra Max 190, Molecular devices Corp., Sunnyvale, CA). The absorbance in the standards was converted into percentage binding (B*i*) with respect to the zero standard (B0). Concentration of the standards was log-transformed and binding data were logit-transformed (logit B*i* = Ln [B*i* $(100\text{-B}i)^{-1}$]). A least squares linear regression was fitted to transformed data and used to calculate the hormone dose in the unknowns.

The assay was evaluated for specificity, sensitivity, and precision. The cross reactivity (specificity) of the T3 and T4 antibodies is summarized in Table 1. To check specificity further, T4 standards were run in plates coated with anti-T3 antibody and T3 standards in anti-T4 coated plates. A binding curve obtained by serial dilutions of a spiked larval extract was compared to the standard curve for each hormone to confirm specificity of the assays. Sensitivity of the T3 and T4 EIAs was defined as the lower limit of the 95% confidence interval of B0. Intraassay coefficient of variation was calculated from the variability between replicate samples assayed within the same plate. Interassay coefficient of variation was assessed by repeated measures of the same sample in different plates and assay dates.

RESULTS

EIA validations

The assays were effective in determining independently both thyroid hormones present in the larval extracts with reasonable precision. The initial assay conditions were approximated by incubations of the tracers with increasing antibody dilutions (Fig. 3.1). The assay was further optimized to reduce nonspecific binding and increase sensitivity.

Table 3.1. Cross reactivity of the anti-T3 and anti-T4 antiserum used in the TH EIA's.

Competitive hormone	Cross reactivity (%) [1]	
	T3	T4
T3	100	< 1.0
T4	< 0.05	100
rT3	~ 0.1	< 0.1

[1] Assayed by direct EIA (manufacturer data)

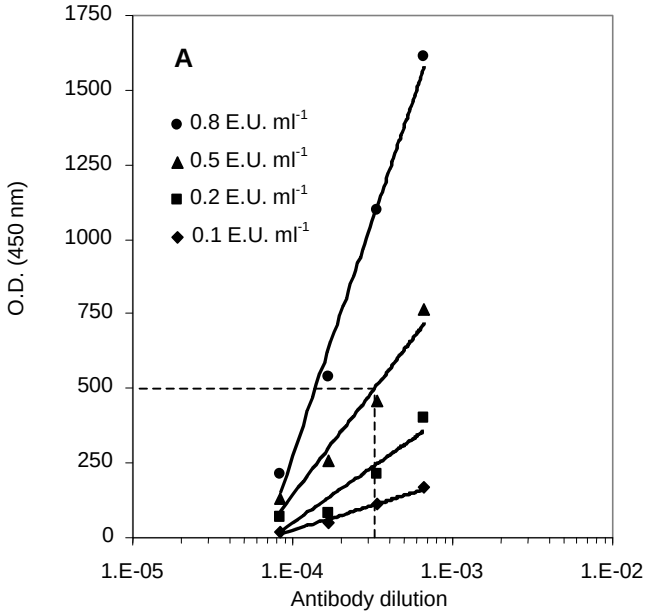
The final conditions for the T3 EIA were a tracer dilution of 0.5 E.U. ml^{-1} and an antibody dilution of 1:3000. Final conditions for the T4 EIA were 0.3 E.U. ml^{-1} of tracer and 1:1500 antibody dilution.

No significant binding was observed by T4 standards run in a plate coated with anti-T3 and T3 standards in an anti-T4 coated plate (Fig. 3.2). The displacement curves obtained with serial dilutions of a pooled homogenate spiked with both T3 and T4 were parallel to that of the hormones' standard curves (Fig. 3.2), further confirming specificity of the assays (Fig. 3.2). Sensitivity of the T3 and T4 EIAs was 2 and 11 pg $well^{-1}$ (n=5), respectively. The effective range of the assay expanding from 20 to 80% binding corresponded to concentrations of 206 to 9 pg $well^{-1}$, and 520 to 30 pg $well^{-1}$, for T3 and T4 respectively. The minimum detectable T3 and T4 dose from 50 mg tissue (80% binding) after factoring the extraction and assay procedures was 0.4 and 1.4 ng g^{-1}, respectively. Intraassay CV at mean T3 binding level of 70-80% was 5.2% (n=60) and interassay CV at mean T3 binding level of 75% was 9.9% (n=5). Intraassay CV at mean T4 binding level of 70-80% was 4.3% (n=60) and interassay CV at mean T4 binding level of 73% was 8.0% (n=5).

Ontogeny of cortisol and thyroid hormones

Red drum grew at a slow rate for the first 2 to 3 weeks porthatching (Fig. 3.3). Coincident with size of settlement in the field, there was an increase in growth rates that remained high through the end of the study at 40 dph when most fish had transformed into juveniles. At the end of the larval period a remarkable rate of body change compared to early larvae was observed which included final development of fins, silvering of the skin and scale development.

Figure 3.1. Tracer-antiserum dilutions test. Symbols identify different tracer dilutions. Dashed lines indicate the conditions chosen of the assay. (A) T3 and (B), T4. O.D. optical density. E.U. Enzymatic Units.



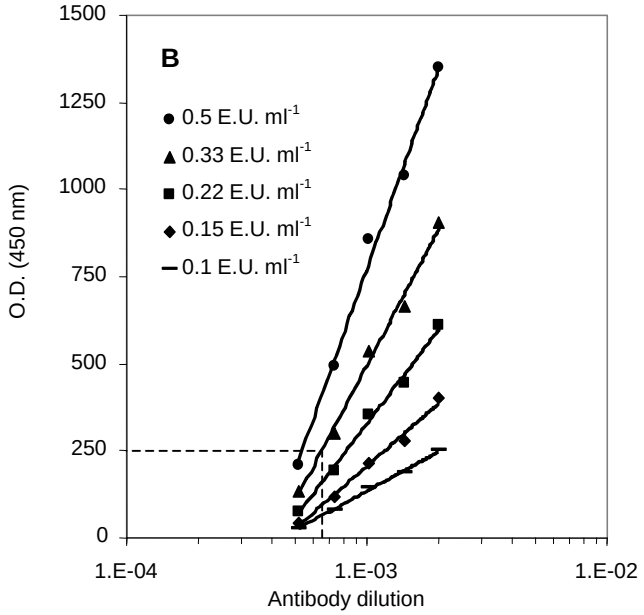


Figure 3.2. (A) Binding curves obtained with plates coated with anti-T3 antiserum using the assay conditions chosen in Fig. 3.1. (B) Log-logit transformations of binding curves shown in A. Equations are given in the figures.

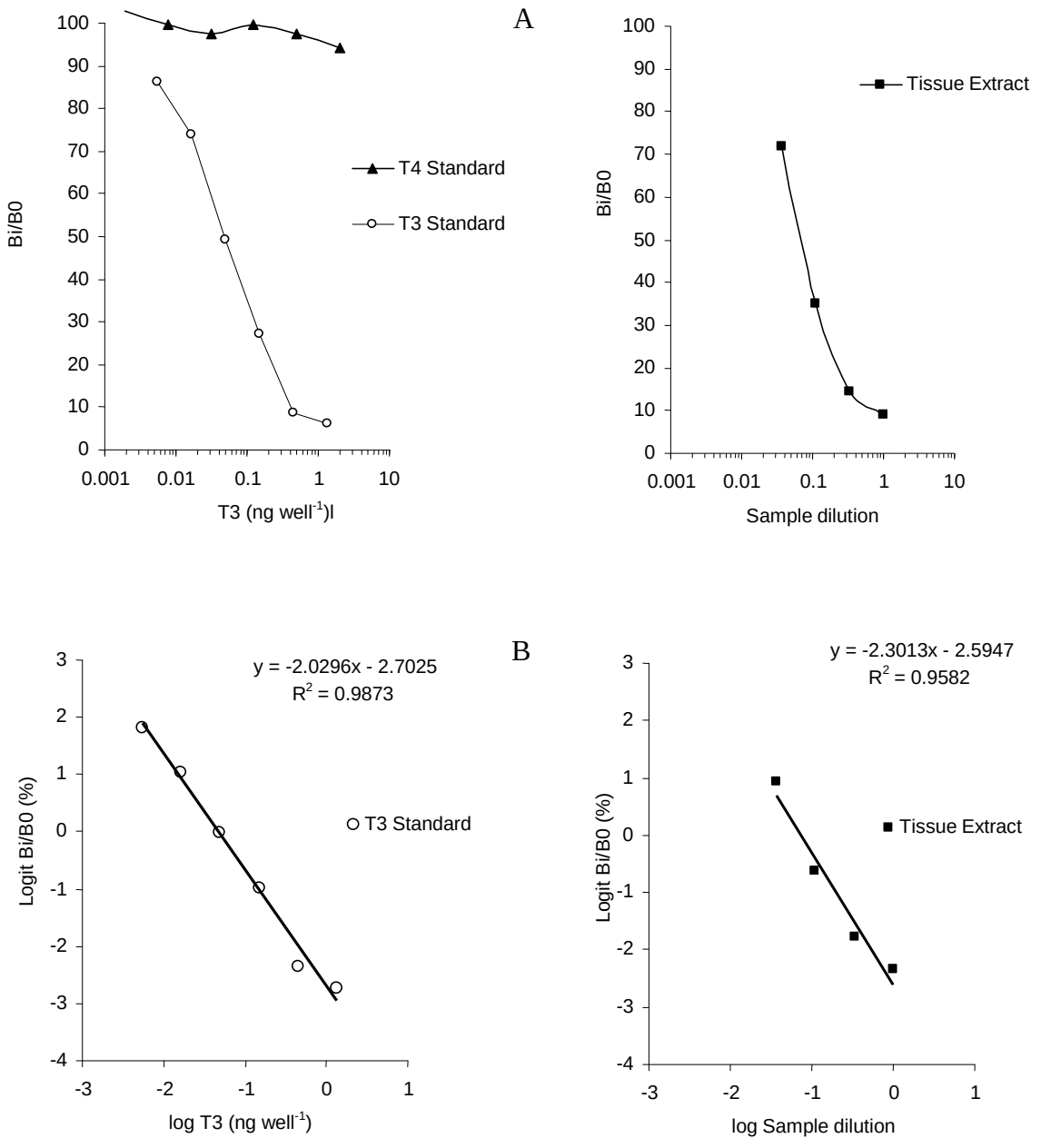


Figure 3.2. (C) Binding curves obtained with plates coated with anti-T4 antiserum using the assay conditions chosen in Fig. 3.1. (D) Log-logit transformations of binding curves shown in C. Equations are given in the figures.

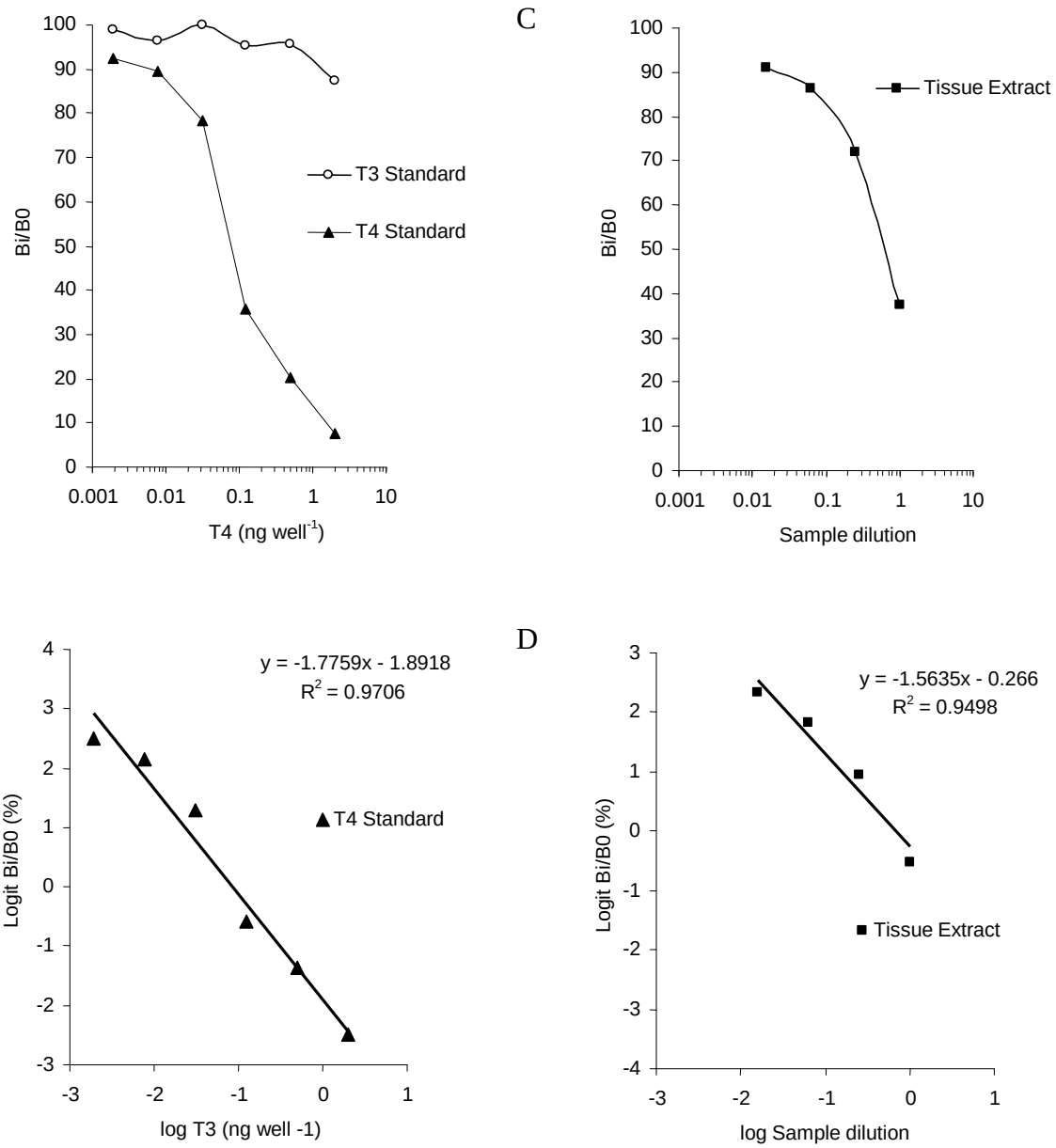
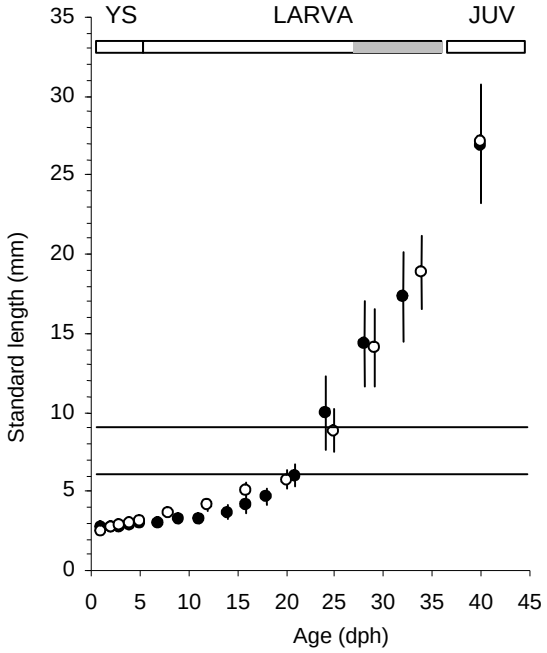


Figure 3.3. Growth in length of two batches of red drum reared at 27 °C. Major developmental periods are given at the top of the graph, yolk-sac larva (YS), larva (LARVA), and juvenile (JUV) periods. Dashed area on larval period indicates the final transformation into juveniles. The two horizontal lines indicate peak settlement sizes in wild red drum. Means ± S.D. (n=50-100).



Cortisol was not detected in eggs (gastrula - tail-bud stage) and 1 dph larvae (Fig. 3.4). Measurable whole body cortisol levels were first found in 2 dph larvae, increased rapidly and peaked right before the end of the yolk-sac period (5 dph). Histological observations of the head kidney did not reveal interrenal cells until 7 dph (Plate 3.1). The early interrenal was organized in a discrete cluster of cells at the base of the head kidney (7-16 dph). In later larvae, interrenal tissue was found along the walls of the posterior cardinal veins (Plate 3.2). Cortisol concentrations sharply declined throughout the larval phase and reached a minimum during the late larva and juvenile stages. Measurable amounts of thyroid hormones were found in fertilized red drum eggs (Fig. 3.5). Whole body content of T4, which is the primary secretory product of the thyroid gland, decreased during the early larval stage. The thyroid gland first appeared at day 3 just before yolk absorption was completed, however whole body T4 content did not increase until after 15 dph. The thyroid contained few small follicles filled with eosin-stained colloid at the time of thyroid differentiation (Plate 3.3). The size of the gland (Plate 3.3 and 3.4) and tissue T4 content steadily increased during larval development. T4 levels showed a 5-fold increase from the minimum premetamorphic levels towards the end of the larval stage. This increase was transient and the levels of T4 markedly declined after metamorphosis. Tissue T3 content was several fold higher than that of T4. Contrasting with the initial T4 decline, whole body T3 concentration significantly increased ($P<0.01$) in 1 dph larvae and peaked in 2 dph larvae. T3 concentrations were elevated again during the late larval phase and during transformation and sharply decreased in juvenile fish.

Diel profiles of cortisol and thyroid hormones

Diurnal fluctuations of whole body cortisol content occurred in both ontogenetic stages (4.5 and 13.9 mm SL) although day-night differences were only statistically significant in the older larvae (ANOVA, $P = 0.048$) (Fig. 3.6). In both cases cortisol

Figure 3.4. Whole body cortisol content during the early development of red drum. Labels are defined as in Fig. 3.3. The inserted graph shows in detail the first 8 dph. Eggs are represented by day 0. Asterisks represent significant differences (*P <0.05 and **P <0.01) with respect to the preceding samples

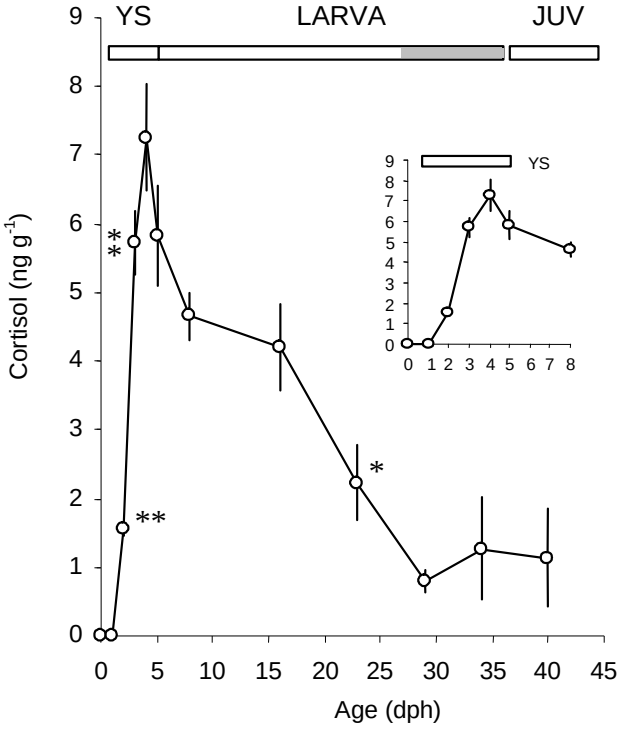
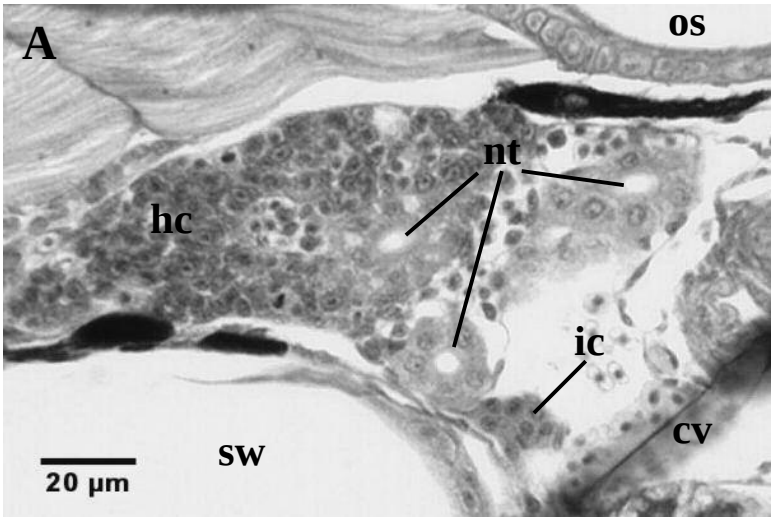


Plate 3.1. Sagittal sections of the head kidney showing the interrenal gland of laboratory-reared red drum. (A) First interrenal detection 7 dph. (B) Interrenal cells of 16 dph larva. ic, interrenal cells; nt, nephric tube, cv, posterior cardinal vein; hc haemopoietic cells; sw, swim blader; os, otolith saccule; pc, pharyngeal cavity; ga, gill arch.



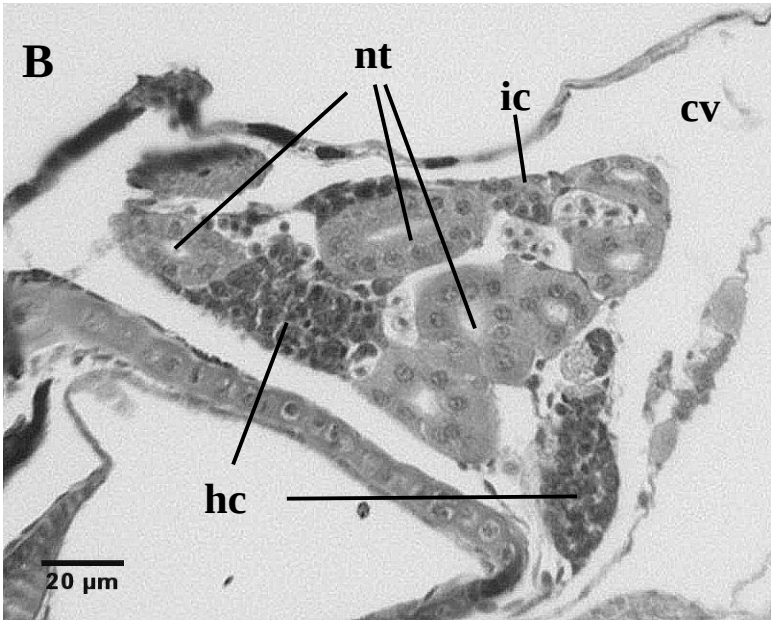


Plate 3.2. Horizontal sections of the left head kidney showing the interrenal gland of laboratory-reared red drum. (A) 21 dph larva. (B) 36 dph transforming larva. Legends are described in plate 3.1.

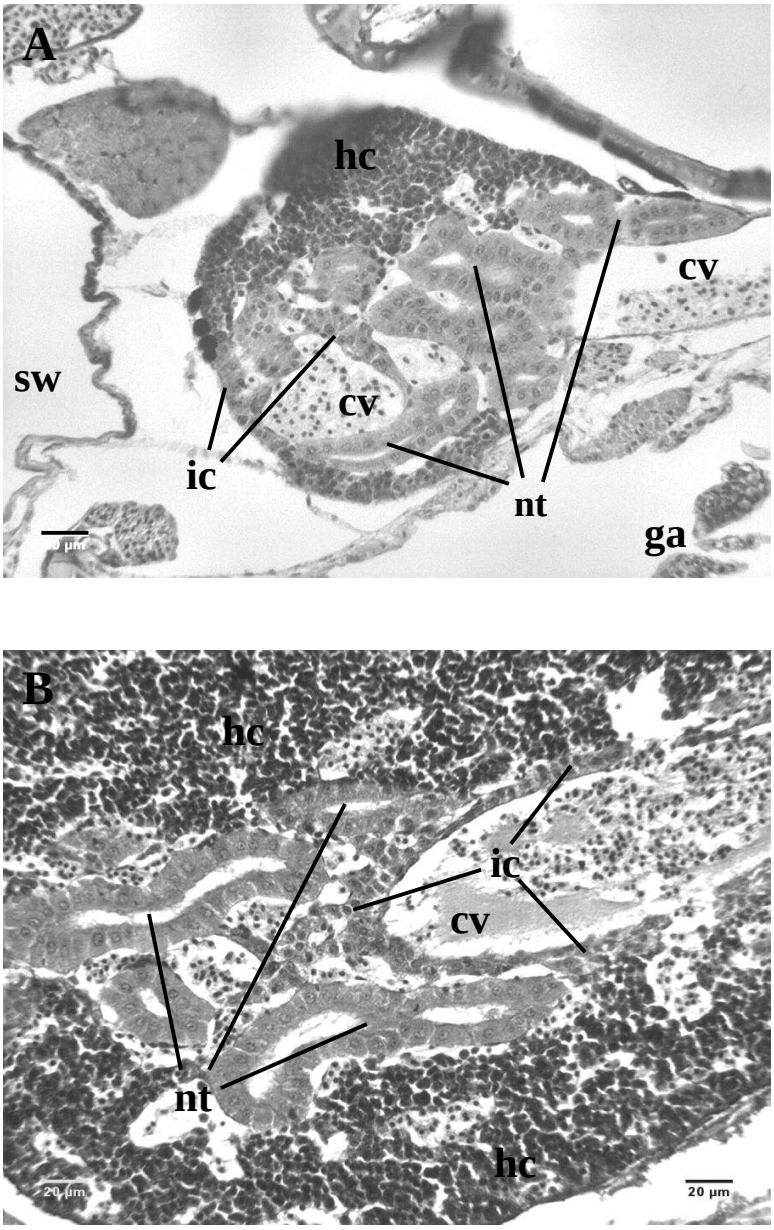


Figure 3.5. Whole body triiodothyronine (T3) and thyroxine (T4) content during the early development of red drum. Labels are defined in Fig. 3.3 and 3.4. Inset shows the first eight days posthatching. Asterisks represent significant differences (*P <0.05 and **P <0.01) with respect to the preceding sample.

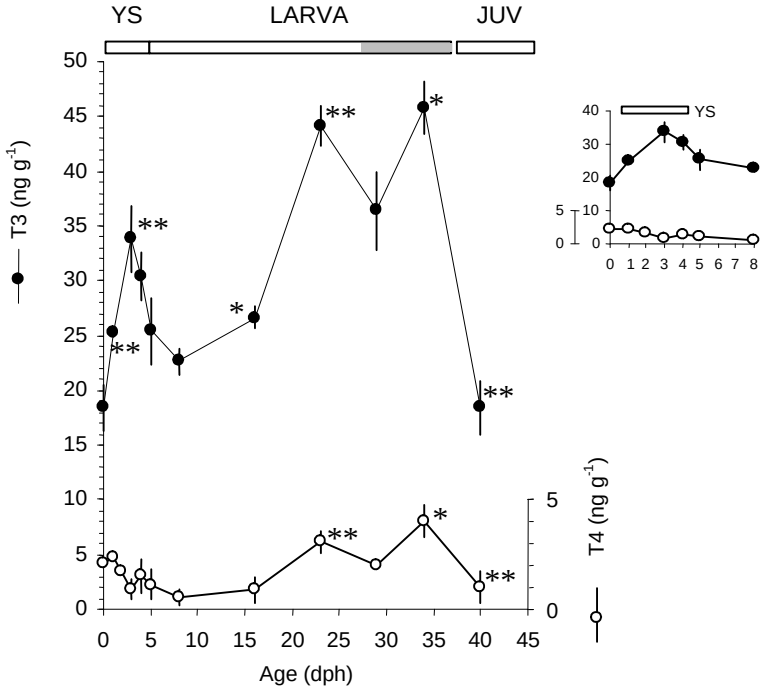
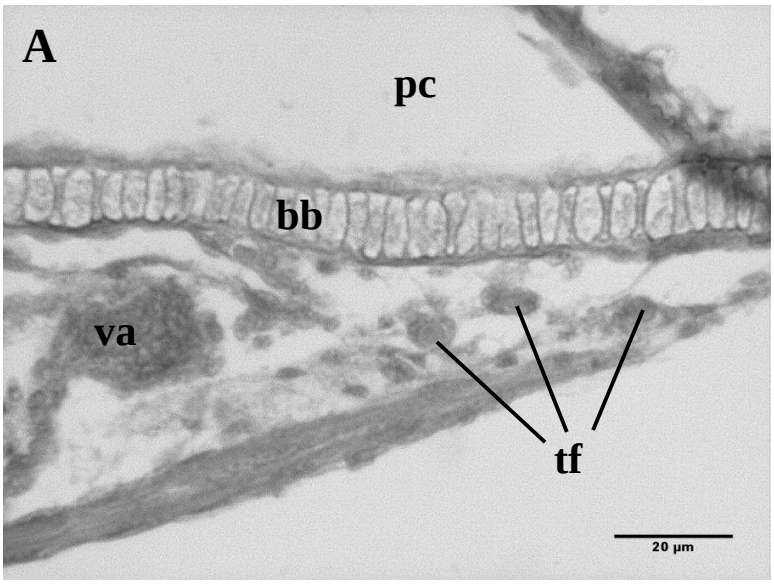


Plate 3.3. Sagittal sections of the thyroid gland of laboratory-reared red drum. (A) First thyroid follicles of yolk-sac larva 3 dph. (B) Thyroid gland of 11dph larva. bb, basibranchial bone; tf, thyroid follicle; pc, pharyngeal cavity; va, ventral aorta: r, retina; ga, gill arch.



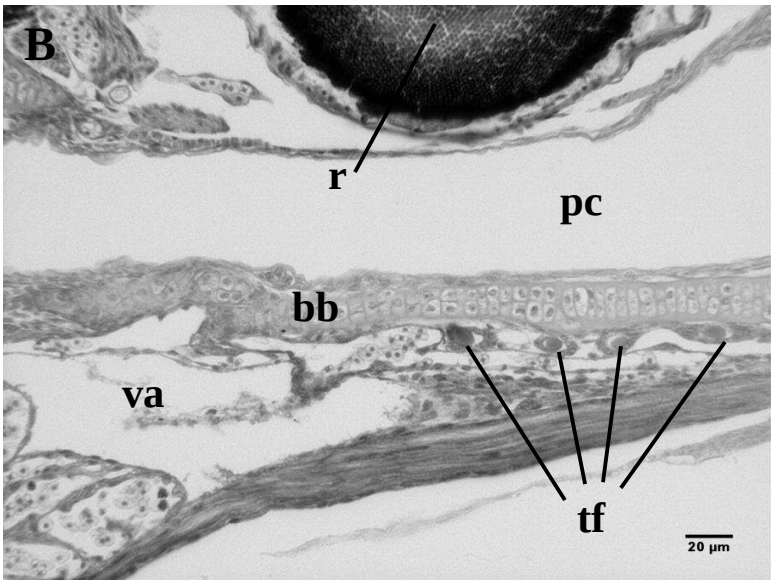
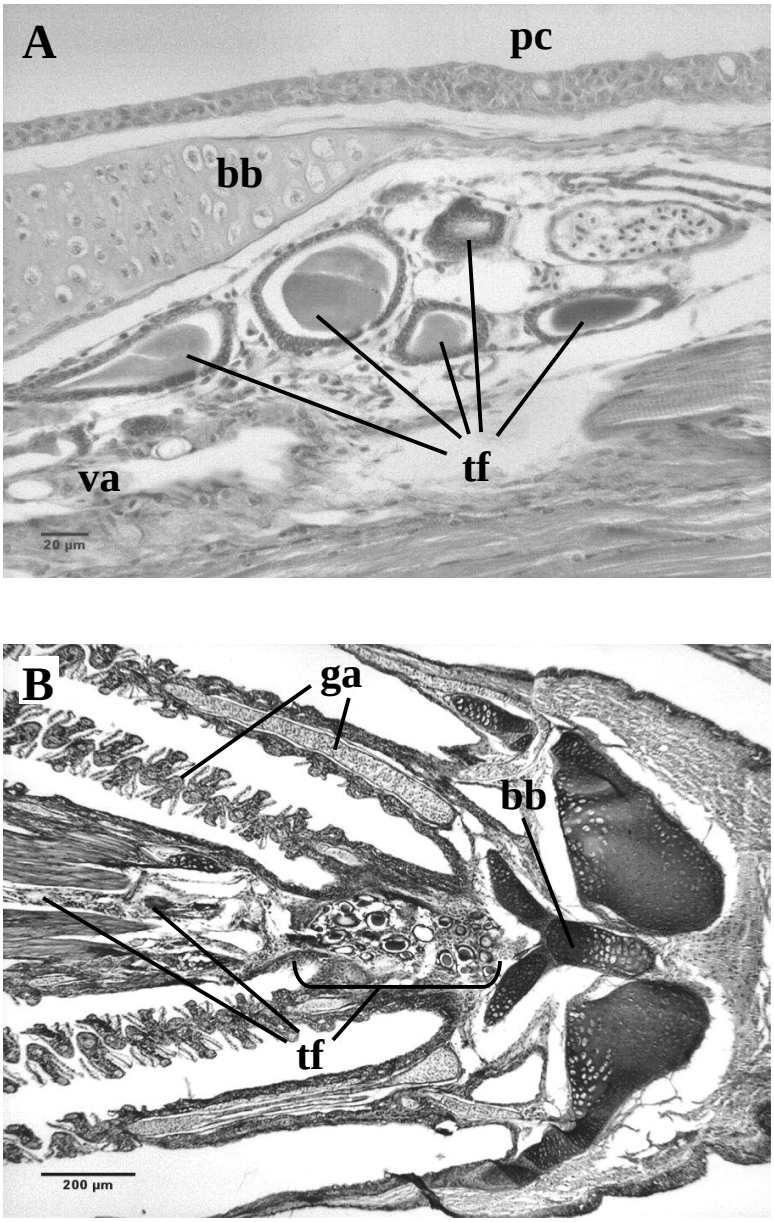


Plate 3.4. Thyroid gland of laboratory-reared red drum. (A) Sagittal section of 21 dph larva. (B) Horizontal section of 36 dph transformation larva. Legends are described in plate 2.3.



appeared to decline during the night with a tendency to increase before dawn. Whole body T3 concentrations showed statistically significant diel changes in the early stage, tending to increase during the day and to decrease after midnight (Fig. 3.7). In older larvae, however, no diel changes in T3 were apparent. In contrast, whole body T4 exhibited a tendency to increase during the day and to drop to its lowest levels at night in both ontogenetic stages examined (4.5 and 13.9 mm SL) (Fig. 3.8).

Cortisol response to environmental stimuli

Handling

The time course of whole body cortisol concentration in response to the acute disturbance (AD) was clearly different between the two ontogenetic stages (7.9 and 8.9 mm SL) tested (Fig. 3.9). Baseline levels were in close agreement with the ontogenetic and diel profiles of whole body cortisol content. Compared to basal levels, the smaller larvae showed only a slight and insignificant increase in cortisol 15 min after AD (Fig. 3.9). Whole body cortisol concentrations declined significantly, below pre-AD levels, 30 min after AD, and quickly dropped well below pre-AD for the entire duration of the 24-h recovery period. In contrast, 9.8 mm SL larvae showed a typical cortisol stress response, a rapid (within minutes) 9-fold increase in cortisol concentration followed by a rapid decrease to the basal level (Fig. 3.9).

Fluctuating environments

Growth rates of fish grown in the TEMP and moderate DO cycles (DO_{mod}) were not different from their respective controls (Table 2). However, growth rate was significantly reduced with respect to the control tanks in the DO_{sev} treatment. No significant differences were found between daily mean levels of cortisol content, consequently all data are presented grouped by treatment and phase. After 10 d of the experiment, cortisol

Figure 3.6. Diel profiles of whole body cortisol in red drum larvae. Alternating light and black bars indicate day and night. (A) 4.5 SL (19 dph) larvae and (B) 13.9 SL (30 dph) larvae. n.d. not determined. The *P*-value for the one-way ANOVA is given. Bars sharing the same letter are not significantly different (*P*>0.05).

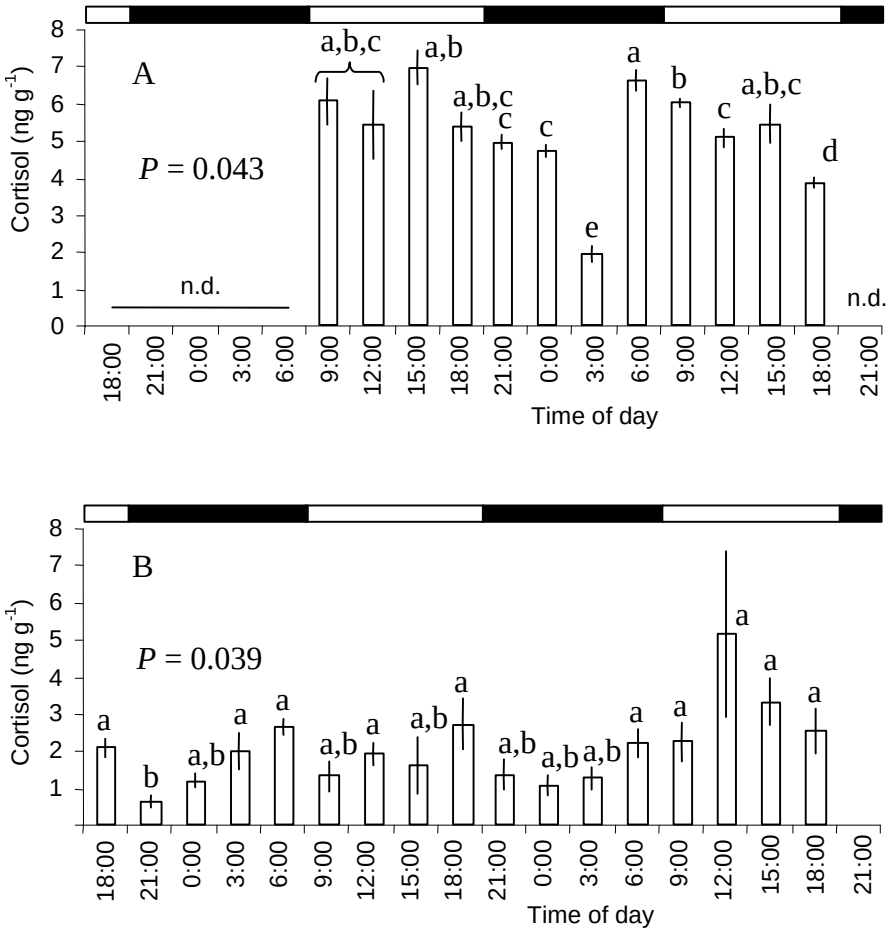


Figure 3.7. Diel profiles of whole body T3 in red drum larvae. Labels are defined as in Figure 3.6. (A) 4.5 SL (19 dph) larvae and (B) 13.9 (30 dph) larvae. n.d. not determined. The *P*-value for the one-way ANOVA is given. Bars sharing the same letter are not significantly different ($P>0.05$).

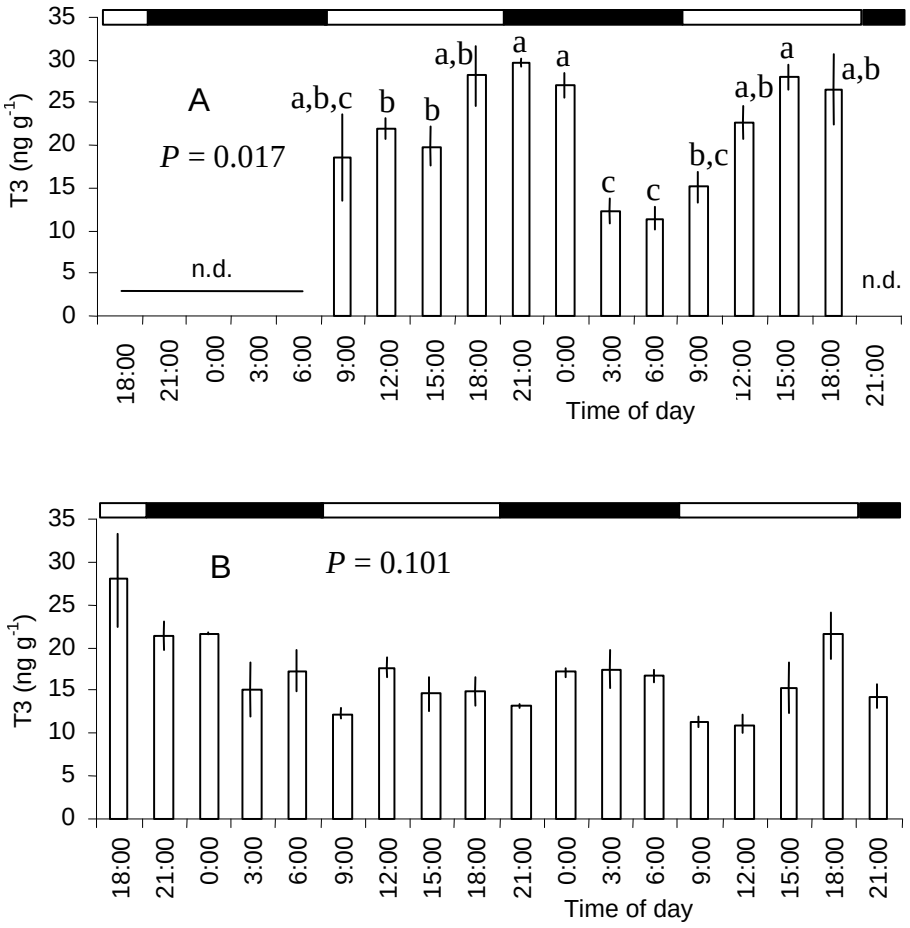


Figure 3.8. Diel profiles of whole body T4 in red drum larvae. Labels are defined as in figure 3.6. (A) 4.5 SL (19 dph) larvae and (B) 13.9 (30 dph) larvae. n.d. non determined. The *P*-value for the one-way ANOVA is given. Bars sharing the same letter are not significantly different (*P*>0.05).

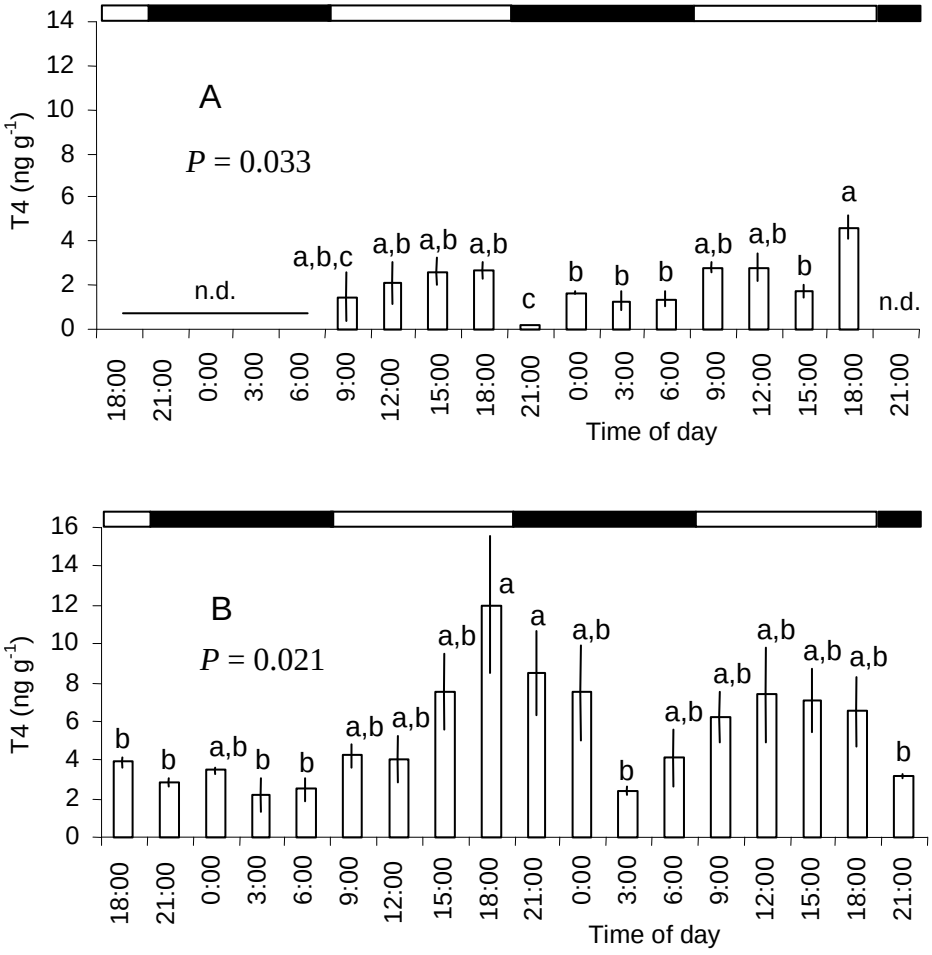
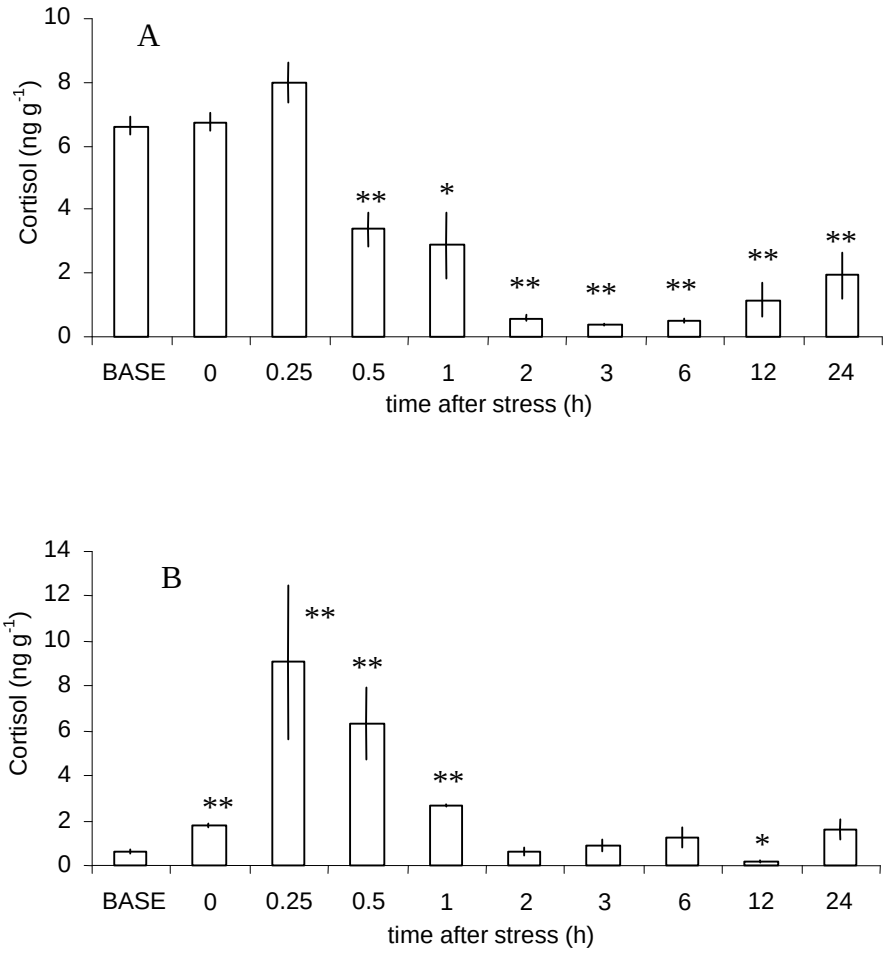


Figure 3.9. Cortisol response after stress in red drum larvae at two ontogenetic stages. (A) Peak settlement size larvae (7.9 mm SL), and (B) late settlers (9.8 mm SL larvae). BASE indicates baseline levels before acute disturbance. Asterisks represent significant differences (*P <0.01 and ** P <0.05) with respect to baseline levels.



content was higher than control larvae in the TEMP treatment larvae, lower than controls in the DO_{mod} and not different from controls in the DO_{sev} treatment (Table 2). By the end of the experiment similar differences in cortisol content in the TEMP and DO_{mod} were seen in the dusk (18:00 h) and dawn (6:00 h) samples but not in the midday samples. Again the cortisol content of larvae in the DO_{sev} trial did not differ from controls even though their growth coefficient was significantly less (Table 2). No stress related changes in cortisol were detected in response to hypoxia in the DO treatments.

Table 3.2. Instantaneous growth coefficient in length (G) and cortisol content of red drum larvae exposed to diel temperature (TEMP) and DO (DO_{mod}, and DO_{sev}) cycles. Means ± SE. Asterisk indicates a significant differences with respect to the control group ($P<0.05$). Larvae were 4.9-5.8 mm SL at the beginning of the experiments; L_f indicates length of the fish (SL, mm) at day 15 of the experiment.

		G (x 10^{-3})				Cortisol (ng g^{-1})		
Experimental interval / day		0-5	5-10	10-15	L_f	0-10	day 15	
Sampling time						*noon*	*noon*	*night*
TEMP	cycles	6.1 ± 0.2	4.9 ± 0.5	3.7 ± 0.4	24.5	3.6 ± 0.6*	4.0 ± 0.6	1.9 ± 0.3* a
	control	6.0 ± 0.2	4.8 ± 0.4	3.6 ± 0.2	23.6	3.0 ± 0.4	3.6 ± 0.5	0.8 ± 0.2 a
DO_{mod}	cycles	6.8 ± 0.0	5.1 ± 0.2	4.2 ± 0.4	22.3	3.2 ± 0.3*	2.2 ± 0.3	4.4 ± 0.5* b
	control	8.0 ± 0.7	5.2 ± 0.5	3.7 ± 0.0	22.4	4.2 ± 0.3	2.2 ± 0.3	6.5 ± 0.8 b
DO_{sev}	cycles	3.8 ± 0.3*	3.0 ± 0.5	4.0 ± 0.5	18.0*	2.6 ± 0.2	2.4 ± 0.3	2.8 ± 0.3 b
	control	4.9 ± 0.4	3.5 ± 0.5	4.4 ± 0.9	21.8	2.7 ± 0.2	2.8 ± 0.4	2.6 ± 0.3 b

(a) sampled at dusk, (b) sampled at dawn. No differences in survival were observed between treatments and control fish under any cycle condition.

DISCUSSION

Differentiation of the red drum thyroid system coincides with final depletion of yolk and initiation of exogenous feeding at a time when well-coordinated sensory, motor and digestive functions are essential for survival. In adult fish, the thyroid produces L-thyroxine (T4) under the stimulation of TSH (thyroid stimulating hormone) from the hypothalamus. T4 is secreted into the bloodstream and converted into 3-5-3'-triiodo-L-thyronine (T3), primarily in the liver by selective outer-ring deiodination (ORD) (Leatherland, 1982; Eales 1985). T3 is thought to be the biologically active thyroid hormone since it shows much greater affinity to specific nuclear receptors and greater potency than T4. However, both T4 and T3 bind to the nuclear thyroid hormone receptor and potentially share similar roles. There is evidence suggesting that tissue deiodination pathways, especially ORD have important regulatory roles in thyroid function (Eales 1985, Eales *et al.* 1993). The relative importance of peripheral (ORD level) and central (TSH level) regulation of thyroid status is still unclear (Leiner and MacKenzie 2003) and may vary with species or ontogenetic status. Increased tissue concentrations of T3 appears in red drum larvae well before the end of the yolk-sac period, while T4, presumably stored in the yolk, decreases. This is suggestive of conversion of T4 to T3 by active ORD in the larvae as early as 1 dph. The site and mechanism of such early ORD is unknown. There are several examples where messenger RNAs of maternal origin are present and actively translated in embryos (Richter 1991; Sundaram *et al.* 2003). The active ORD observed here could be of maternal origin since histological observation of red drum larvae at this stage revealed only undifferentiated body structures. Red drum is remarkable among most marine teleosts (Tagawa 1990) in having T3 present in much higher concentrations than T4 (Leiner and MacKenzie 2003). The early but transient

activation of ORD is also quite remarkable, and both suggest a very important role of peripheral regulation of thyroid status in red drum.

A second surge of thyroid hormones was observed in the late larval period when the larva morphology is undergoing final structural changes prior to the juvenile period. This second thyroid activation involves both T4 and T3 and the initial rise in hormone content occurs at about 3 weeks posthatching, the time when larvae in nature begin to settle out of the plankton into nursery grounds. In teleosts, TH increases are coincident with important developmental and ecological events such as: onset of active feeding (review in Tanaka *et al.* 1995), emergence in chum salmon (Tagawa and Hirano 1989), inshore migration in black sea bream (Tanaka *et al.* 1991), freshwater migration in Japanese seabass (Perez *et al*. 1999), or settlement in red sea bream (Kimura *et al.* 1992). Interpretation of these observations is difficult since they do not provide evidence for a direct role of TH in these ecological transitions. However, settlement relies on morphological and behavioral changes to the new habitat that are partially mediated by TH and steroid hormones as first suggested by laboratory studies on flounder (Miwa *et al.* 1988; Tanangonan *et al.* 1989; de Jesus *et al.* 1990: Inui *et al.* 1995). TH administration induces precocious metamorphosis; and antithyroid treatment delays metamorphosis-related changes and results in abnormally large larvae in flounders (Okada *et al.* 2003; Gavlik *et al.* 2002; Solbakken *et al*.1999; Yamano, *et al*.1991). Analogous results are obtained in non flatfish species such as coral trout (Dody *et al.* 2002), red seabream (Hirata 1989), and Pacific treadfin (Brown and Kim, 1995) which show much less dramatic morphological transformations than flounders. Red drum also do not experience dramatic morphological changes, but similar adjustments would be very valuable if they “preadapt” (Specker 1988) their metabolism to exploit nursery grounds more efficiently.

Enzyme immunoassays for measuring TH have multiple advantages over radiometric methods that use strong radioactive tracers, especially those that emit gamma radiation such as Iodine-125, which is commonly used in TH RIA. The main advantage is the elimination of expensive and time-consuming management of radioactive wastes. The tracers used in the EIAs have a shelf-life of about a year which compares favorably with the 3 to 4 weeks effective usage period of radioactive counterparts. The sensitivity obtained in the assays was comparable to that of some RIAs and is reliable enough to measure T3 and T4 levels from as little as 50 mg wet weight tissue sample. The specificity obtained with the present antibodies was adequate for quantification of both TH from the same homogenate without interference as demonstrated by the non-significant reaction of the spiked extracted sample with the T3 standard in the anti-T4 coated plate and vice versa. Inter- and intrassay CVs were considered adequate, however they can probably be reduced further if automated washing methods are employed.

The interrenal gland is difficult to recognize during early development in red drum. The head kidney is initially located under the notocord, anterior to the swim bladder. A series of scattered cells surrounding the nephric tubules is present as early as 4-5 dph, however these cells could not be positively identified as interrenal cells in hematoxylin and eosin stained sections (Takashima and Hibiya 1995). Mature interrenal cells have spherical, dark staining nuclei with distinct nucleoli, and eosinophilic cytoplasm arranged in one to several clusters associated with the posterior cardinal veins and within the hematopoietic parenchyma. Interrenal cells cannot be identified in most fishes until some days after yolk absorption, however whole body cortisol apparently increases before that time (Inui *et al*. 1994, Perez *et al.* 1999). In red drum larvae whole body cortisol increases and peaks, well before yolk absorption. The initial increase in cortisol may indicate the first activation of corticosteroidogenic pathways as suggested by

positive immunoreactivity of embryos and early larvae of Asian sea bass (*Lates calcarifer*)) to antibodies raised against corticosteroidogenic enzymes (Sampath-Kumar *et al.* 1996).

Unlike red drum larvae, surges in cortisol concentration during the early ontogeny of Japanese flounder (de Jesus *et al.* 1991; Inui *et al.* 1994) and Japanese seabass (Perez *et al.* 1999) precede those of thyroid hormones. In the laboratory cortisol has a synergistic role with TH in flounders (de Jesus *et al.* 1990) and pacific threadfin (Brown, and Kim, 1995) promoting development-related changes. Similar effects of TH and cortisol are well documented in prematurely-born mammals, suggesting a conserved mechanism across vertebrates. Cortisol is synthesized by direct stimulation of interrenal cells by the pituitary hormone ACTH, which is in turn, controlled from the hypothalamus by corticotrophin releasing hormone (CRH). Synthesis of cortisol is precisely regulated because cortisol, as well as many other steroid hormones, diffuses freely across biological membranes. Like TH, cortisol actions are mediated by specific intracellular receptors and involve differential expression of specific target genes that are ultimately responsible for the specific actions of the hormones. It is probable that ACTH is expressed in the pituitary of red drum larvae at the time of initial cortisol increase as has been suggested recently for Japanese flounder (Estevez *et al.* 2001). At the time of settlement CRH is being expressed, as suggested by the integrated cortisol stress response to acute environmental stimuli. It was interesting to see the lack of a strong cortisol increase following the stress administration in small larvae compared to larger larvae. Smaller larvae already had elevated whole body cortisol content compared to their larger counterparts. Older larvae had probably attained the levels characteristic of the late larva and juveniles periods. Barry *et al.* (1995) suggested the presence of a stress hyporesponsive period during the early ontogeny of rainbow trout (*Oncorhynchus*

mykiss) similar to that described in mammals (DeKloet *et al.* 1988). The hyporesponsive period may help larvae avoid the deleterious and permanent effects that cortisol may have on the neural organization of the brain (DeKloet *et al.* 1988). This lack of response may have an adaptive significance for red drum if the hyporesponsive period coincides with settlement in nature. During settlement larvae are first exposed to rapidly changing environmental conditions that may result in significant stress-related increases in cortisol levels above the already high baseline levels.

Only the prolonged hypoxia treatment resulted in reduced growth rate compared to controls. The reduction in growth rate was limited to the first two sampling intervals probably reflecting a more severe effect of DO limitation in smaller individuals (5.0-15.0 mm SL). This result indicates a greater vulnerability of red drum larvae to environmental stressors during the initial settlement period. In spite of the growth retardation, no differences were found in whole body cortisol in the reduced growth treatments with respect to controls, suggesting that growth retardation was not associated with cortisol-mediated stress. Interestingly these fish did not show the diel changes in cortisol content that fish in controls, moderate DO, and temperature treatments did. Chronic cortisol release could have deleterious effects on metabolism, and repetitive response to nocturnal hypoxia could result in maladaptive responses that compromise survival. Red drum could be programmed not to respond to these brief nocturnal hypoxia events; such an adaptation would avoid the cost of starting compensatory responses when the conditions are likely to improve later in the day.

Fish exposed to environmentally-realistic temperature and DO cycles grew as fast as control groups but may have wider amplitudes in whole body cortisol cycles than their controls, as suggested by the differences found between samples collected at noon and dusk. The direction of the difference with respect to controls was positive (greater

cortisol content in cycled fish) when fish were sampled at dusk and opposite when sampled at dawn. This observation agrees with the general pattern of cortisol fluctuation observed in the diel experiments, in which cortisol concentration rose from relatively low nocturnal values at dawn and then declined at dusk. Cortisol may have greater amplitude cycles under moderate environmental cycling and this may serve to coordinate temporal patterns of other physiological activities in the fish. The actual mechanisms connecting direct physiological reactions to environmental stimuli and the interaction of directive environmental factors with metabolism may involve many and complex physiological pathways. However, system-wide signaling through the endocrine system probably brings about final adjustments and coordination of the physiological status of the fish. Through these responses, environmental cycles may affect an individual's performance and distribution, and then change population- or even community-level dynamics.

Study of hormones during settlement provides information on programmed developmental processes and crucial adjustments to environmental conditions of the nursery. Settlement and subsequent growth of red drum larvae while using nursery grounds is central to recruitment, and it is likely that a number of evolved responses to directive environmental factors are reflected in the phenotype (physiological response) during this period. An appreciation of the factors that affect the magnitude, duration and timing of hormone production and other physiological changes caused or regulated by hormones is important for proper understanding of the interconnection between environment and hormones.

In summary, red drum have the capacity to activate T3 and cortisol production during the yolk-sac period; the functionality of the interrenal and thyroid axes is probably achieved before settlement; fish growth may be reduced in habitats with transient but severe hypoxia, however no cortisol stress response is activated in response to hypoxia;

and environmentally realistic temperature and DO cycles do not result in a cortisol-mediated stress response by larvae.

CHAPTER 4: SETTLEMENT DYNAMICS AND RECRUITMENT POTENTIAL OF RED DRUM (SCIAENOPS OCELLATUS) LARVAE TO A SUBTROPICAL SEAGRASS NURSERY: A LENGTH-BASED APPROACH COMBINED WITH ENCLOSURES STUDIES

ABSTRACT

Larval red drum settle into shallow seagrass meadows. Previous field surveys have shown clear environmental differences between edge and core seagrass that may result in differential settlement and habitat value. The aims of this study were to determine the distribution of young red drum between edge and core seagrass and to assess the relevance of spatial and temporal variation in nursery conditions to cohort-specific growth and mortality patterns (G':Z ratios) of settled larvae. Field collections were conducted twice weekly in the fall of 2002 and 2003 at two sites located at core and edge areas of a seagrass bed in Aransas Bay, Texas. Dominant modes (cohorts) in length-frequency distributions were followed over time to calculate growth and mortality rates. Size-defined cohorts arrived in seagrass beds from early September to late October. Based on larval lengths, larvae appeared to settle at the deep-edge of the seagrass meadow and accumulate in the meadow's interior, or core seagrass. During the active settlement period densities at the edge were 2– 10-fold lower than at the core. The pulsed influx of settlers coupled with high mortality rates indicate that population size is largely dependant upon supply of settlers and larval mortality immediately after settlement. Growth rates were high with no seasonal trend. Mortality was substantial and variable, influencing the recruitment potential index (G':Z). No seasonal trends in G':Z were observed and most cohorts appeared to contribute to recruitment. Cages at edge and core areas stocked with hatchery-reared larvae failed to demonstrate consistent habitat differences although some experiments had reduced mortality at the edges. Growth of

cultured red drum followed that of wild counterparts during settlement and subsequent recruitment to the nursery. Edge seagrass areas may be very important in determining successful settlement since they provide the first and crucial contact with the nursery habitat.

INTRODUCTION

Many estuarine-dependent organisms have evolved complex life histories that take them through ontogenetic shifts in habitat utilization. Recruitment is often linked to variability in settlement of early life stages to benthic nursery habitats (Underwood and Fairweather 1989; Sale 1990; Heath, 1992; Jones *et al.* 1999; Searcy and Sponaugle 2001). A complex suite of ecological processes operates during this transition and subsequent use of the nursery habitat. Differences in ecological responses of fish populations to biotic, abiotic and landscape characteristics of the nursery create site- and time-specific variation in growth and survival that can affect the nursery value for a species, which is thought to play a critical role in determining the relative strength of a year-class (Sogard 1992; Beck *et al*. 2001). Consequently an understanding of the factors contributing to nursery habitat quality would provide insight into the mechanisms that determine recruitment.

Seagrass meadows vary from relatively deep, patchy habitat at the deep edge to shallow, more uniformly vegetated habitat at the core. Previous environmental surveys of the shallow seagrasses and marsh edges used by red drum larvae in the Aransas Estuary have shown considerable environmental fluctuations (see chapter 2). Diel cycles in temperature can be as high as 6 ºC, and dissolved oxygen (DO) can attain hypoxic conditions (2-4 mg O_2 l^{-1}) just before dawn and supersaturated levels at noon. Additionally, drops in temperature and salinity associated with the passage of frontal

weather systems can be substantial in these shallow environments. Environmental surveys demonstrated that the deeper seagrass areas bordering the meadows are substantially more stable and severe hypoxic events are rare. Laboratory studies showed that growth and survival is not affected by normal diel variations in environmental conditions within the range found in seagrass meadows used by red drum larvae (see chapter 2). However, these microhabitat differences may result in differential habitat utilization that may affect growth and/or survival during settlement (Breitburg 1994) and therefore determine differences in habitat quality (Gibson 1994; Beck *et al.* 2001; Holbrook and Schmitt, 2003) for red drum. In other words, does recruitment potential vary in space? No information is available on the use of deeper (1.2 m) areas of seagrass during settlement and whether these areas may serve as temporal refuge for settled fish during episodic hypoxia events.

Growth rates and mortality rates during the early life stages are important vital rates that determine recruitment variability in fish populations (Houde 1987, 2002). The ratio between instantaneous growth rate in weight (G') and mortality rate (Z) has been used to evaluate cohort performance (Houde 1989; Houde and Zastrow 1993). A cohort in which Z is greater than G' is loosing biomass over time and this may lead to recruitment failure, thus these ratios can be used to estimate recruitment potential. For most marine species cohort biomass declines during the early larval stages (Z > G'). It is clear that at some point the biomass must increase for successful recruitment to take place. Recruitment potential will depend on when during the ontogeny the threshold Z = G' is crossed. The estimates of survival potential (Z:G') or growth potential (G':Z) of larval cohorts during the postsettlement period can serve as an index with which to compare quality of nursery habitats and ultimately habitat value for the early stages of fishes.

Several analytical tools have been developed to create life tables of early life stages from which to estimate vital rates. Most methodologies currently used are built upon a proxy of age or past environmental experience laid down permanently in fish otoliths with some correlation with size. This information is used to derive age-length relationships. These tools have grown in sophistication and accuracy over the past few decades (Campana *et al*. 1997; Francis 2003) and are providing extremely important insight into the dynamics of larval fish populations. However, there are still some difficulties in their application that are mainly related to validation of the age-length estimates for wild fish and the considerable labor and expense associated with sample processing (Campana and Neilson 1985; Campana and Stevenson 1992). A common approach to circumvent these concerns is to derive age composition from length-frequency data using an age-length key based on a subset of the actual sample. In this type of approach, the variability in length-at age is often ignored. An alternative approach is to estimate vital rates directly from length-frequency data. This method greatly reduces costs, parameter estimates are based directly on the length data rather than the size-at-age estimates, and it utilizes all available observations. The length-frequency approach is commonly used to approximate growth and mortality estimates in catch-at-age models for fisheries stock assessments and a number of software packages are available at no cost (FAO, Rome). A drawback of the length-frequency approach is the need for relatively large sample sizes to reliably represent the age-structure of the population in the length-frequency distribution. Also this method is only applicable to those species that show the production of distinct larval cohorts over the spawning season.

Red drum (*Sciaenops ocellatus*) spawn in the early fall along shallow coastal waters of the western Gulf of Mexico. Larvae enter the estuaries and settle into seagrass and marsh-edge habitats (Holt *et al*. 1983 and 1989; Rooker and Holt 1997; Rooker

1998b; Stunz *et al.* 2001). Red drum settles at sizes that range from 4 to 11mm standard length (SL) (peak settlement size is 6-8 mm SL) (Herzka *et al.* 2002) with densities of up to 11.5 m^{-2} in shallow seagrass habitats in the Aransas Estuary (Rooker et al. 1998). Brown (2002) modeled the ingress of particles through the only inlet to the Aransas Estuary, and found that most "recruits" to the estuary will arrive in a few discrete pulses that are correlated with the actual passing of red drum larvae through the inlet. Together these observations suggest that a length-frequency approach would be adequate for the study of red drum settlement dynamics and recruitment.

Field studies were designed to determine if larval distributions of, growth and mortality were uniform regardless of location or physical characteristics of the habitat. The null hypotheses were: A) Settling red drum larvae do not select particular microhabitats within a homogeneous seagrass meadow, and B) Microhabitat-related differences in environmental condition in space and time do not affect recruitment potential. Fish collections are included to evaluate habitat use, and to determine if recruitment potential varies in time or with differences in environmental conditions. Enclosure studies using cultured fish are used to approximate the relative importance of environmental factors to the overall growth and mortality experienced during the post-settlement phase (Duffy, *et al.* 1997; Planes and Lecaillon 2001), since these estimations can seldom be derived from fish surveys (Pepin 1991; Houde 2002).

The goals of this research were to: (1) determine if red drum select distinct depth-related microhabitats within a continuous seagrass environment during settlement; (2) evaluate differences in growth and mortality with respect to microhabitat differences using caging experiments; and (3) derive growth and mortality estimates from red drum surveys to calculate recruitment potential of natural cohorts after settlement into the nursery.

MATERIALS AND METHODS

Study site and general sampling procedures

Fieldwork was conducted in a shallow seagrass meadow in the Aransas Estuary (Texas) between mid October through late November 2002, and late September to early December in 2003. The general area was surveyed before the initiation of the study, on a day where the bottom was clearly visible, to identify two stations (deep-edge and core) within a large shoal grass (*Halodule wrightii*) meadow. The deep-edge (EDGE) station was defined by a rectangle measuring 220 by 90 m oriented parallel to and outside to the 1.5 m depth contour. The core (CORE) station was a 200 m square adjacent to the EDGE station on the shallow bank of the meadow. A beam-trawl (1 m wide, 0.25 m tall) fitted with a 500 µm mesh was used for all red drum collections. Sampling at the EDGE station was conducted by towing the beam-trawl along 50-90 m transects from a shallow-draft boat. Tows were perpendicular to the depth contour and measured with a WAAS-enabled global positioning system (GPS). In 2003 a video camera was fitted to the frame of the beam-trawl for the EDGE tows to determine the percentage of time the gear was in vegetated areas and calculate the actual amount of seagrass habitat sampled. The CORE station was sampled by pulling the beam trawl by hand using a 20-m rope (transect 20 m) after a visual assessment of the bottom to ensure that only seagrass was sampled. After removing drift algae and coarse material, the catch was immediately preserved in 5% buffered formalin. Red drum larvae were picked out in the laboratory and photographed along with a reference scale (used for calibration) and the standard length (SL) of each larva was measured to the nearest 0.01 mm using ImageJ 1.30v software (http://rsb.info.nih.gov/ij).

Environmental surveys

Data sondes were deployed continuously for 7 and 11 weeks (2002 and 2003, respectively) at the edge and core stations throughout the settlement season. Temperature, dissolved oxygen concentration (DO), pH, conductivity, and water depth were recorded at 30-min intervals by multiparameter water quality data sondes (YSI Incorporated, OH, USA) placed within the seagrass canopy. Sondes were checked and data downloaded weekly to ensure proper working condition, calibrate sensors and prevent data loss. Water quality readings were compared with discrete observations made throughout each deployment to assess data quality. Additionally, DO sensor condition was logged simultaneously with DO readings. DO data that did not meet the expected quality criteria were excluded. Temperature records for Aransas Bay were obtained from the Texas Coastal Ocean Observation Network automated monitoring program (http://dnr.cbi.tamucc.edu/wiki/TCOON), station DNR ID: 015.

Field collections

Diel movement study

EDGE and CORE stations were sampled twice daily (DAWN and NOON) at two separate times during the 2002 settlement season. The first was conducted in mid-October when maximum settlement was expected. The second one was conducted in early November when settlement of new fish was completed. For each sampling period, five replicate tows were conducted at each site and time of day for two consecutive days (8 collections total). Fish were immediately preserved in 5% formalin and later measured. A length-frequency distribution (1-mm size interval) was calculated for each combination of date, time and station. Analysis of variance (ANOVA) was used to test for differences in density and fish size between EDGE and CORE stations. Separate two-sample

Kolmogorov-Smirnov tests were used to compare length-frequency distributions between DAWN and NOON collections at each station and collection date. All statistical comparisons were conducted using Systat v10.0 software, (SPSS Inc., Chicago, IL) and a significance level of $P < 0.05$ was used for all comparisons.

Modal Progression Analysis

Three to 12 replicate tows were collected twice weekly for 6 and 11 consecutive weeks at the EDGE and CORE stations in 2002 and 2003 respectively. There were 10 collections (trips) in 2002, with equal effort in all trips (5 tows per station). In 2003 the total number of trips was increased to 18, and due to lower fish densities, the effort was increased to 12 tows per station, but on five trips only 6 tows were collected. Fish were sorted, measured, and for each trip, separate length-frequency histograms (1-mm length classes) were calculated for the EDGE and CORE stations. The fish were then pooled by trip and new length-frequency histograms were calculated at 0.5-, 1.0- and 2.0-mm length classes. These were used to identify the length class that best described the underlying age structure in the sample. Next, the length-frequency distributions were separated into a series of normally distributed components (assumed cohorts) according to dominant modes using the Bhattacharya and Maximum Likelihood Method (NORMSEP) routines available in FiSAT II v1.0.0 software (http://www.fao.org/fi/statist/fisoft/fisat) (see Gayanillo *et al.* 1996, 1997). The Bhattacharya method is based on the fact that the logarithmic derivative of the normal distribution (slope) decreases linearly (Bhattacharya 1967). Therefore assuming the length-frequency distribution is composed of several overlapping normally distributed components, it is possible to separate them by looking for negatively sloped linear intervals in the plot of the first-order differences of the natural logarithms frequencies ($\mathbf{Ln\ N} = \text{Ln } N_{n+1} - \text{Ln } N_n$; slope) on the length midpoints. The linear regression is transformed back into a normal distribution of known mean

length and standard deviation along with an estimate of the size (abundance) of the component. This first component is then removed from the data set. The result is a new length-frequency distribution in which the first component (presumed cohort) has been eliminated, to which the method can be reiterated. Since the method relies on several subjective decisions the analysis was accepted only if the linear regression coefficient, and the separation index (SI*) between successive components were greater than 0.75 and 2.0 respectively.

$$*SI= (L_{i+1} - L_i)/ [(S.D._{i+1} + S.D._i)/ 2]$$

were L_i is the mean of the component *i*, L_{i+1} is the mean of the *i* +1 component, $S.D._i$ is the standard deviation of the component *i* and $S.D._{i+1}$ is the standard deviation of the component *i* +1

To further limit bias, a second analysis using NORMSEP was conducted on the same length-frequency data. This method uses the (weighted) sum of squares of deviations between a designated model and observations, which is analogous in concept to the goodness-of-fit used in linear regression analysis. NORMSEP works as an iterative process that needs an initial "guess" of the component's mean length and number of components present in the series. The output of the Bhattacharya analysis was used as the initial guess. NORMSEP also provides a mean length, standard deviation and a abundance estimate. Finally the output of both analyses were compared and the resultant components (assumed cohorts) were averaged to obtain the final estimate of number of components present, and each component's mean length, standard deviation and size for the different trips.

The final step of the MPA was the linking of means. Mean lengths of identified components were plotted separately against days and effective degree-days (DD_{eff}, see next section) and the points (judged to correspond to the same cohort) connected to

obtain growth trajectories. Two persons linked the means separately and only the points in which both linkages matched were assigned to a particular cohort and subsequently used to estimate cohort-specific growth and mortality rates.

Instantaneous cohort-specific growth coefficients (*G*) were calculated from an exponential growth model described as

$$SL_t = SL_0 \, e^{(G \times t)}$$

where SL_0 is the SL (mm) at t =0 and SL_t is the SL at time t.

The model was fit to the data using the non-linear regression routine in Systat v10.0 software, (SPSS Inc., Chicago, IL). The coefficient of growth in length (G) was converted to wet-weight-specific growth coefficient (G') using a power correlation between wet-weight (W) and standard length (SL) ($W = 10^{-05} \, SL^{3.164}$, n= 251, R^2= 0.99) derived from caged and laboratory fish.

Cohort-specific mortality rates were calculated from population abundance data provided in the MPA. Abundances were log-transformed and the data smoothed using kernel density estimator (uniform smoothing function with a bandwidth of 2, Systat v10.0 software, SPSS Inc., Chicago, IL). Transformed abundance data were plotted separately against days and DD_{eff} and a linear regression fitted to the descending limb of the plot. Points located to the left of the maximum value were considered to represent cohorts not yet fully recruited and consequently were not included in the mortality estimates. Instantaneous mortality rates (*Z*) were calculated from an exponential decay model described as

$$N_{DDeff} = N_0 \, e^{(-Z \times t)}$$

where N_0 is the number of individuals settled at t =0 and N_t is the abundance at time t.

Survival potential for each cohort in which paired instantaneous growth and mortality estimates could be made was calculated as the ratio of weight-specific growth

to instantaneous mortality (G':Z) and used as an index of recruitment potential of the cohorts.

Growth rate (G), mortality estimates and G':Z ratios were calculated with respect to days (d) and to DDeff.

Cage experiments

Experimental animals

Several batches of red drum larvae were raised at the University of Texas Marine Science Institute, Fisheries and Mariculture Laboratory (FAML), from early August thru late November 2002 and 2003. Fertilized eggs were obtained from natural spawns of temperature- and photoperiod-conditioned broodstocks (Arnold 1988) kept at FAML and at the CP&L Marine Development Center, Texas Park and Wildlife Department, Corpus Christi, TX. Eggs were stocked (50-100 eggs l^{-1}) in 150-l conical tanks. Egg incubation and larval rearing were conducted at 26-27 °C, 27-31 psu in well-aerated filtered seawater using flow-through (25 ml min^{-1}). Only batches with hatching rates greater than 95% were used. Larvae were co-fed rotifers and a formulated larval feed (Kyowa B; Kyowa Hakko Kogyo Co., Ltd. Tokyo). In 2004, frozen brine shrimp (*Artemia* sp.) enriched overnight with *Isochrysis galbana* (UTEX LB 2307) was used in addition to formulated feeds (Proton 2 INVE AQUACULTURE Inc., Ogden, UT). Ten to 12 days posthatching larvae were transferred to 350-l cylindrical tanks from which they were removed as they reached the target size for the experiments.

Cages and experimental set up

Cages (1 m x 1 m footprint) were open to the bottom and had 1.2-m tall meshed side panels (1.1-mm mesh) to allow for natural water exchange (Herzka *et al.* 2001). A refinement of the design, consisting of the addition of an outer frame and raised side

panels, was employed at the EDGE station in 2003 to allow deployments at depths up to 1.6 m. After setting the cages their bottoms were swept with a 1-m wide, square dip net (1.5-mm mesh size) to remove possible predatory fishes. No attempt was made to clean small sized crustaceans and other benthic organisms from the cage. Multiparameter water quality data sondes (YSI Inc., Yellow Springs, OH) deployed at each cage site monitored temperature, DO, salinity, pH and depth at 30 min interval

Larvae were stocked into cages deployed within the EDGE and CORE stations at sizes corresponding to the range of sizes found at seagrass habitats (see Table 4.3). Two and three replicate cages per station were used in 2002 and 2003, respectively. Larvae were randomly divided into clear plastic bags placed within insulated Styrofoam boxes and the bags sealed after the addition of oxygen. All boxes were then transferred to the field and half were used to stock the cages. The bags were introduced into the cages for 45 min and water was partially exchanged twice before the fish were released into the cage after confirming the lack of dead larvae. The number of fish stocked varied inversely with the size of the larvae being tested and ranged from 12 to 50 individuals. No food was provided to the caged fish since the design allows the fish to feed on natural foods (Herzka *et al.* 2001). The remaining fish were brought back into the laboratory and used to stock the control tanks. Two control groups were kept in the laboratory through the end of each caging experiment. Each control group was run in triplicate. One control group was fed (CTRL), while in the second group food was withheld (sCTRL). Environmental conditions in the control tanks were adjusted to approximate observed field conditions (temperature, DO and salinity) at the time.

Hypoxia was defined as those DO concentrations below which red drum experience oxygen limitation. The effect of temperature was factored in the calculation of the limiting oxygen concentration (LOC) threshold by setting a sliding scale between 18

and 28°C and constant thresholds above and below this temperature range. Temperature range and LOC were defined following LOC values published by Neill *et al.* (1990 and 2004) for red drum juveniles. Salinity and feeding were not factored in these calculations.

$LOC = 2.0 \text{ mg } O_2 \text{ } l^{-1}$ if $T \leq 18$ °C

$LOC = -3.4 + (0.3 \times T)$ if $18 < T < 28$ °C

$LOC = 5.0 \text{ mg } O_2 \text{ } l^{-1}$ if $T \geq 28$ °C

Sampling, growth and mortality estimates

In 2002 all fish from two bags were sacrificed with a lethal dose of MS222 upon returning to the laboratory and measured to estimate initial fish size. In 2003, all fish going into a particular cage or tank were measured under anesthesia (0.04 g l^{-1} MS222) before being stocked, and survivors were measured again at the completion of the experiment. Fish were recovered from the cages with 1-m wide, square dip nets as described for the removal of predators. A cage was considered empty after no fish were captured in three consecutive sampling strokes.

Since a major objective was to compare growth and mortality of red drum under contrasting environmental conditions in laboratory, cages and field conditions, all growth and mortality estimates were calculated on effective degree-days (DD_{eff}) (Fry, 1971; Kamler, 1992) to maintain for a time axis that allowed comparison among different studies independent of temperature differences. DD_{eff} was calculated by subtracting an empirically derived biological zero (temperature at which red drum growth is zero, T0= 8.7 °C) (see chapter 2) from the thermal sums starting on the date when the data sondes were first deployed (14 September, $DD_{eff} = 0$).

Instantaneous growth coefficients (G) were calculated from an exponential equation described as

$$G = (LnSL_f - LnSL_0)/ DD_{eff}$$

where SL_0 and SL_f are the standard lengths at the beginning and end of the experimental interval, and DD_{eff} represents effective degree-days.

Control cage deployments of 1-, 6-, and 24-h duration were conducted throughout the study with different sized larvae to determine recovery rates. No significant differences in recovery were found between the three acclimation periods. However, smaller larvae had significantly lower recovery (average 92%) than larger fish (>16mm SL) in which recoveries were always 100%. Recovery from laboratory controls was 100%. Instantaneous mortality rate (Z) was calculated from an exponential decay model in which a correction for recovery efficiency was introduced. The equation was,

$$Z=[(Ln(N_0/N_f)-(LnN_0/N_c)]/ DD_{eff}$$

where N_0 and N_f are the number of individuals at the beginning and end of the experimental interval, and Nc is the mean number of larvae recovered from the control cages (Fuiman 1994).

Data analysis

Analysis of variance (ANOVA) was used to compare average densities between years. Density estimates were Ln-transformed prior to analysis to meet the assumption of equal variances. Growth and mortality trajectories of individual cohorts were compared within years by testing for differences in slopes using analysis of covariance (ANCOVA) using size as the covariate. The between-years comparison was conducted on the G' and Z estimates using two-sample Student's *t*-test. The recruitment potential estimates (G':Z) were compared within years by one-way ANOVA and between years by a two-sample Student's *t*-test. Separate one-way ANOVAs were conducted to test differences in growth and mortality among control treatments, cages and natural cohorts for each caging experiment. Natural cohorts included in each analysis were those coincident in time with the caging experiment. Caging experiments that did not coincide with a natural cohort

were not included. The analysis was followed with pair-wise comparisons (Tukey HSD test) at a level of significance of $P < 0.05$. All statistical tests were computed with Systat v10.0 software, (SPSS Inc., Chicago, IL).

RESULTS

Environmental surveys

Water temperature, DO, salinity and depth records showed important temporal and spatial variation throughout the red drum settlement period (Figs. 1, 2, and 3). Mean temperature during the first half of the study was slightly higher in 2002 (26-30 ºC) than in 2003 (24-27.5 ºC). In 2002 water temperature started to drop in mid-October, 2 weeks earlier than in 2003, coincident with the first fall frontal system (front) that swept the area. A mild late season characterized 2002 relative to 2003, where a succession of strong fronts, in combination with extremely low tides (Fig. 4.3) brought the lowest daily temperature below 10 ºC on several occasions in 2003. Two record temperature drops were recorded on 24 and 28 November, in which water temperature dropped from 26.3 to 6.9 ºC and from 25.0 to 8.1 ºC, respectively (Fig. 4.1). These extreme events coincided in time with the last caging experiment of the 2003 season (C2003-5). Where data were available, the amplitude of the diel oscillation in temperature was 1 to 2 ºC larger at the core station during the second half of the study. Water DO concentrations showed a marked diel oscillation with nocturnal decreases often falling below the incipient oxygen limitation for red drum (hypoxia, 5.0 mg O_2 l^{-1}) (Neill *et al.* 1990, 2004) at dawn (Fig. 4.2). Nocturnal hypoxia events were suppressed during the low salinity period that occurred at the end of the 2002 recruitment season. The hypoxia events were always more prominent at the core station. Salinity was similar between years during the first

half of the study and ranged from 16 to 27.5 psu in 2002 and 20 to 30 psu in 2003 (Fig. 4.3). A remarkable drop in ambient salinity was registered in 2002 due to intense runoff caused by two tropical storms that hit the area on 25 and 31 October (data not shown). Consequently salinity in the last third of the 2002 season was in the range of 4 to 8 psu for about 3 weeks, which is extremely unusual in the study area. Coincident with the low salinity period, an algal bloom was observed in the study site. This period was also coincident with the last two caging experiments of the 2002 season (C2002-2 and C2002-3). Overall, both years had comparable environmental conditions early in the season, however contrasting environmental conditions between years developed in the second half and were characterized by extremely low salinity and relatively mild temperature in 2002, and typical salinity conditions but severe temperature drops in 2003.

Figure 4.1. Water temperature records taken at 30-min intervals for the EDGE (solid grey) and CORE (black) seagrass areas, and the Aransas Estuary (dotted grey) during the 2002 and 2003 red drum recruitment season. Horizontal bars symbolize the duration of caging experiments. Mean size (SL, mm) and number of fish stocked per cage (n) in each experiment are given over the bar. Black diamonds represent the dates of red drum collections.

Figure 4.1.

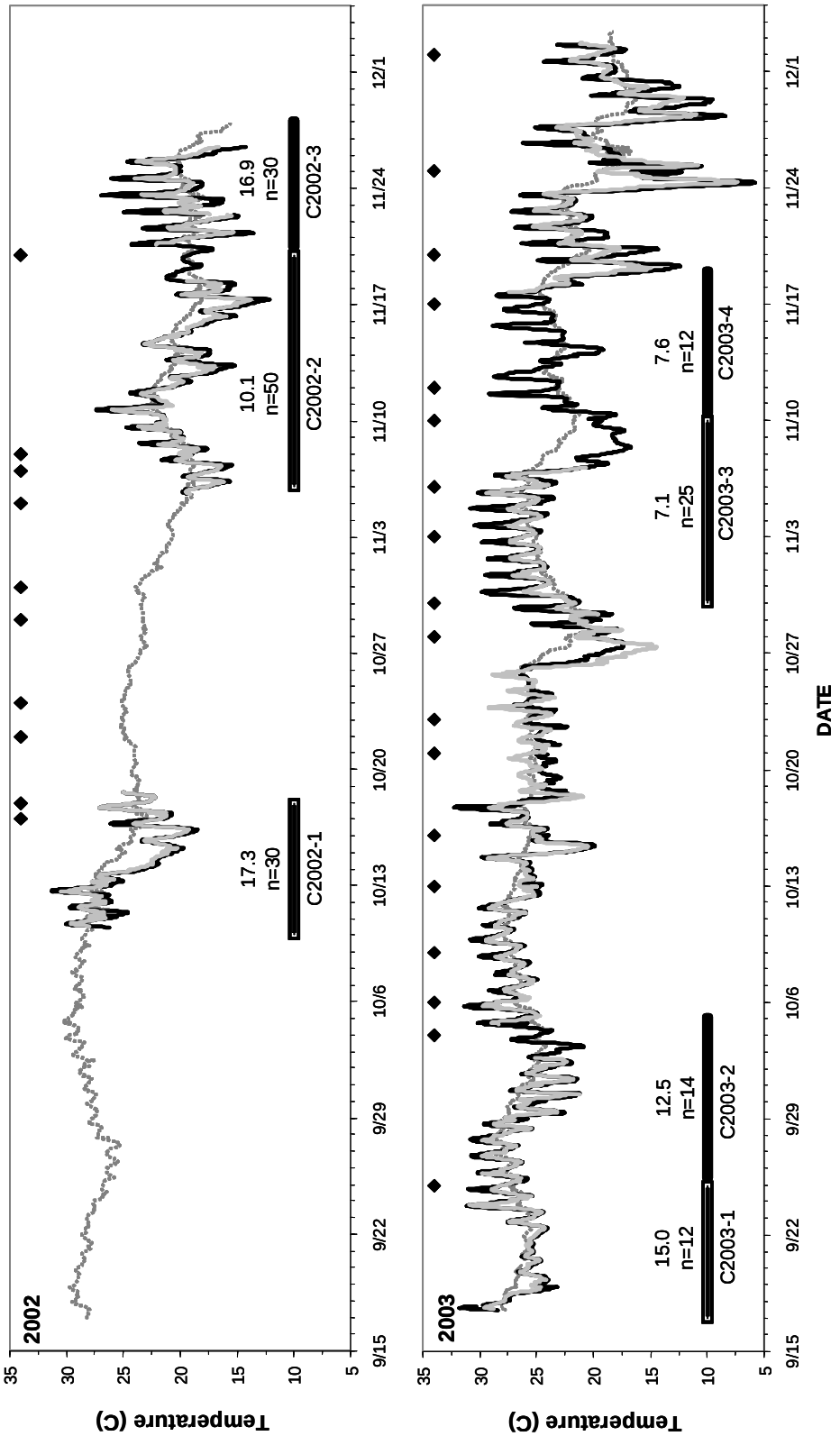

2002
2003
Temperature (C)
DATE
9/15
9/22
9/29
10/6
10/13
10/20
10/27
11/3
11/10
11/17
11/24
12/1
35
30
25
20
15
10
5
17.3
n=30
C2002-1
10.1
n=50
C2002-2
16.9
n=30
C2002-3
15.0
n=12
C2003-1
12.5
n=14
C2003-2
7.1
n=25
C2003-3
7.6
n=12
C2003-4

Figure 4.2. Dissolved oxygen records for the EDGE and CORE seagrass areas during the 2002 and 2003 red drum recruitment seasons. Labels as in Fig. 4.1. Horizontal line indicates limiting oxygen concentration at 28 °C for juvenile red drum.

Figure 4.2.

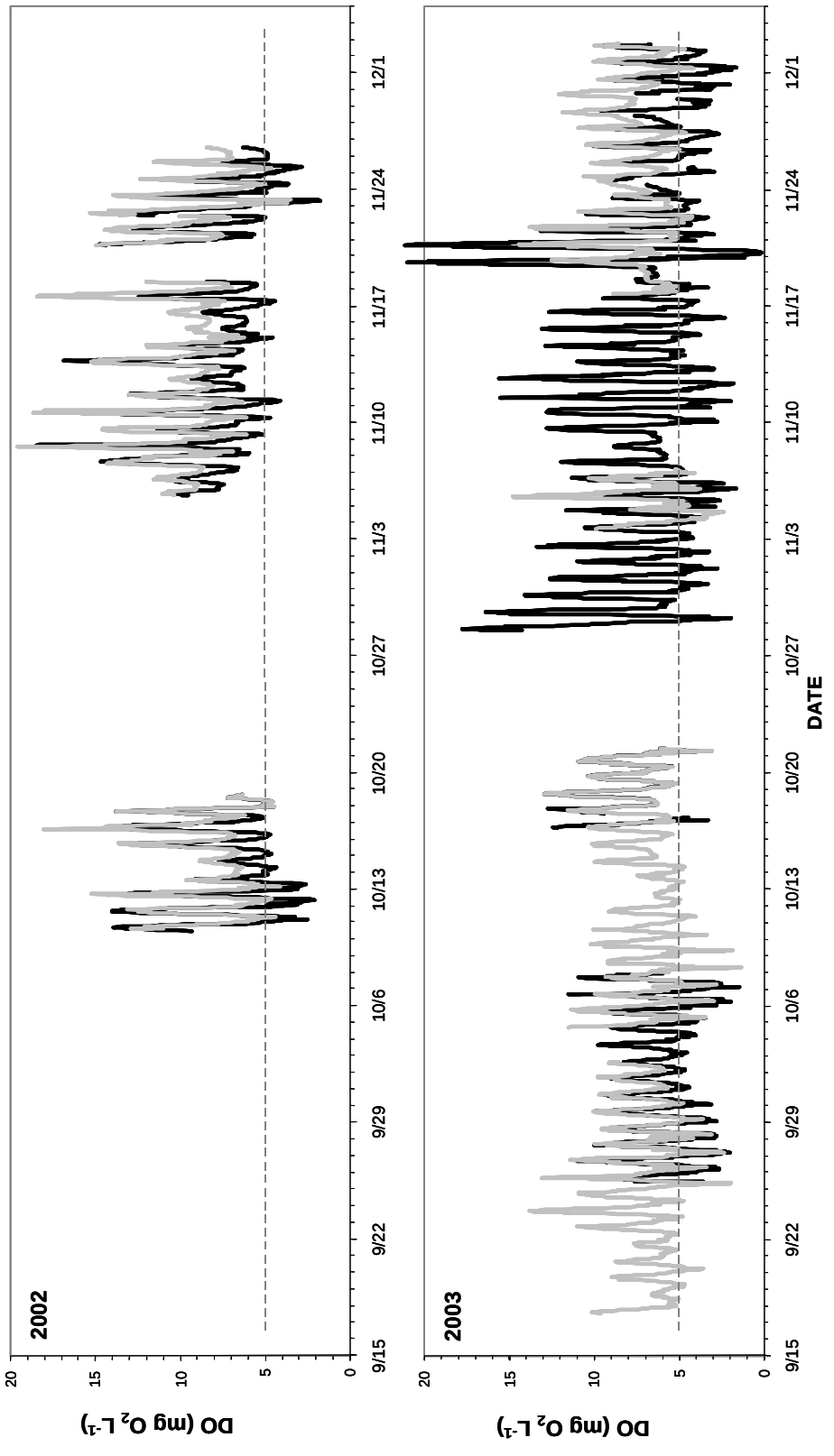

2002
2003
DO (mg O2 L-1)
DATE
0
5
10
15
20
9/15
9/22
9/29
10/6
10/13
10/20
10/27
11/3
11/10
11/17
11/24
12/1

Figure 4.3. Salinity and depth records for the EDGE and CORE seagrass areas during the 2002 and 2003 red drum recruitment seasons. Labels are applied as in Fig. 4.1. Depth is presented on the right side axis.

Figure 4.3.

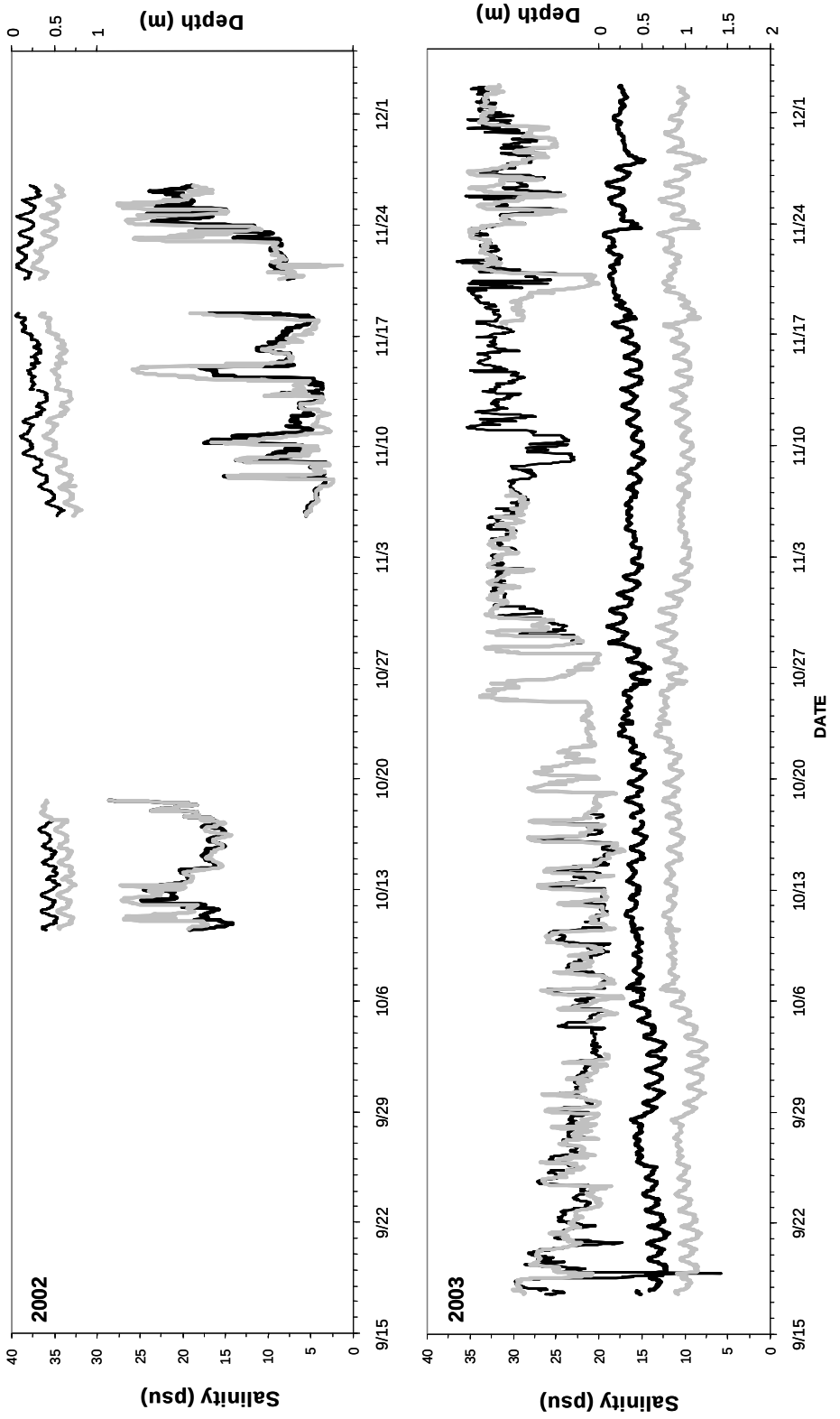
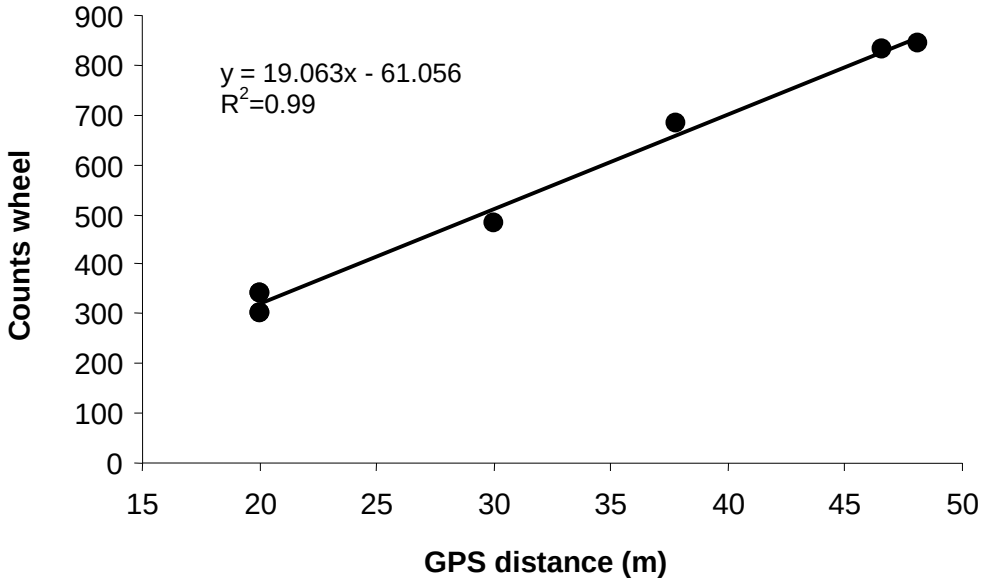

2002
2003
Salinity (psu)
Depth (m)
DATE
9/15
9/22
9/29
10/6
10/13
10/20
10/27
11/3
11/10
11/17
11/24
12/1

Figure 4.4. Relationship between GPS calculated distance and measuring wheel estimations performed simultaneously for the same transect on six sample tows.



Field collections

A novel methodology was devised to quantitatively sample in the deep-edge seagrass areas. Transect length was estimated using a WAAS-enabled global positioning system (GPS) and was validated with a measuring wheel attached to the frame of the beam-trawl. Both estimates described a straight line over the range of distances sampled, validating the use of GPS alone to conduct the surveys (Fig 4.4). Video recordings made during the actual tows indicated that the area of seagrass sampled in the deep-edge ranged from 96 to 100%. The contribution of bare sediment was considered minor and no corrections of the density estimates were made.

Diel movement study

During collections DO dropped to 61-68% saturation on the first two sampling dates, and to 72% saturation on the last sampling date (Table 4.1). Diel temperature changes were within the normal 3-6 ºC range characteristic of shallow seagrass habitats (Table 4.1). The first two collection dates fortuitously coincided in time with an intense settlement event that allowed for a detailed assessment of fine scale settlement patterns. No differences were found in density (Table 4.2), or in length-frequency distribution between DAWN and NOON collections (Fig. 4.5). The 6- and 7-mm size classes strongly characterized the EDGE station while 9- and 10-mm size classes dominated in CORE samples (Fig. 4.5). The 8-mm size class marked the transition from EDGE to CORE (Fig. 4.5). Red drum were present in CORE samples at densities 3- to 5-fold greater than in EDGE samples. At the edge, the density increased through the end of the 2 days sampling and the length-frequency distribution apparently shifted up by one size class in 19 h (noon 17 October, to dawn 18 October). During the second diel experiment, fish density at the CORE was very low (0.24 m^{-2}) and no fish were caught at the EDGE (Fig. 4.6). Due to the lack of fish at the edge station the second day collection was not conducted.

Red drum larvae were absent from planktonic surveys collected during the week before at the only inlet of the Aransas Bay (S.A. Holt, unpublished data*). Likewise, no differences between dawn and noon length-frequency distributions were detected.

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Regular collections

Mean standard length and length-frequency distributions of fish caught at the EDGE were always shifted towards smaller sizes (ANOVA, P <0.05) (Fig. 4.7). The exception was the large collection of 28 October 2003, in which no differences were found between EGDE and CORE seagrass (P = 0.887). In 2002, catch rates showed a steep left limb that peaked at 6- 7 mm in EDGE samples and at 8-9 mm in CORE samples. In 2003, the length-frequency distributions of EDGE and CORE caught fish peaked at 7-8 mm (SL) and 8 mm, respectively. Smallest individuals captured in both CORE and EDGE stations were 4.5-5.0 mm SL.

For both years, density was always lower at the EDGE compared to the CORE station and very few red drum were collected at the EDGE station in the surveys conducted late in the season. Densities (per tow) ranged from 0.0 to 0.4 m^{-2} at the EDGE and from 0.9 to 2.8 m^{-2} at the CORE in 2002, and 0.0 to 0.2 at the EDGE and from 0.2 to 2.1 m^{-2} at the CORE in 2003. Mean red drum density at the core station was 1.8 m^{-2} for the first 4 collections in 2002, representing a 4- to 5-fold increase in abundances compared to the same period in 2003. A sudden drop in density was observed after the onset of the low salinity event in 2002, which resulted in comparable low density of settlers between years towards the end of the study period.

Table 4.1. Temperature, DO, and salinity conditions during the diel collections.

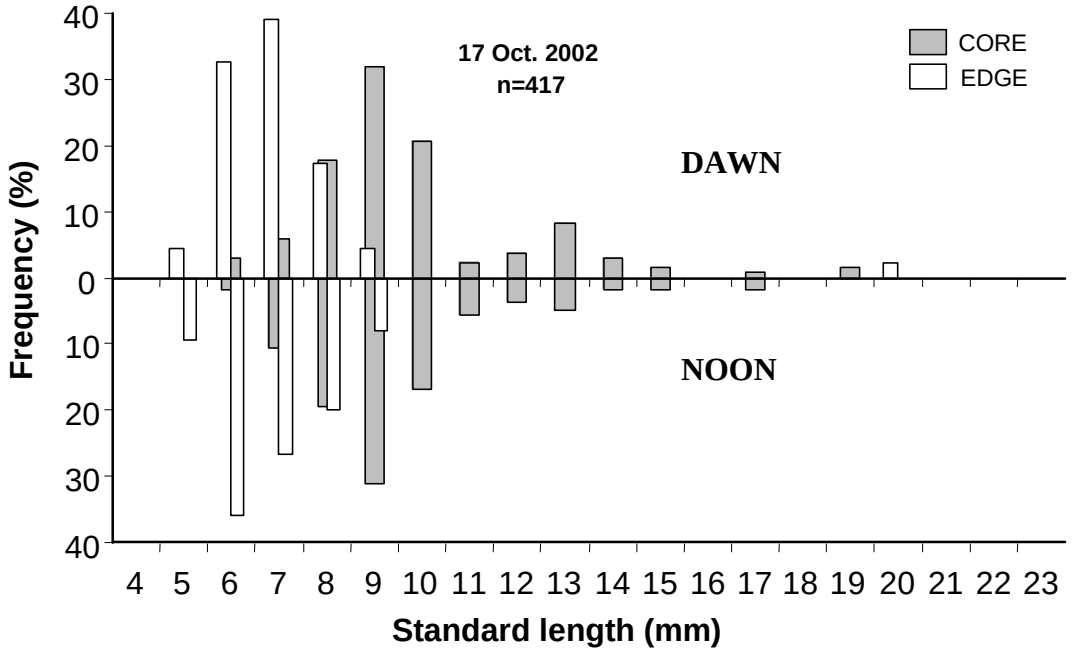
Collection date	Station	CORE		EDGE	
	Time	Dawn	Noon	Dawn	Noon
17 Oct. 2002					
Temp. (C)		20.7	15.6	22.0	25.5
DO (mg O_2 L^{-1})		5.5	11.4	6.7	12.1
DO (%)		68	152	85	170
SAL (psu)		15.8	19.0	15.8	24.5
18 Oct. 2002					
Temp. (C)		22.2	24.8	22.5	25.2
DO (mg O_2 L^{-1})		4.8	6.5	6.5	8.2
DO (%)		61	93	85	117
SAL (psu)		20.1	24.0	19.3	29.3
8 Nov. 2002					
Temp. (C)		17.7	23.4	18.3	21.9
DO (mg O_2 L^{-1})		6.7	15.9	8.9	16.0
DO (%)		72	190	96	188
SAL (psu)		3.6	3.9	3.9	3.8

Table 4.2. Mean density of red drum larvae (no. m^{-2}) per sampling station and time of day in a shallow seagrass meadow. Numbers represent mean abundance ± SE (no. m^{-2}) of red drum collected in five replicate tows. Values sharing same letter were not statistically different (P>0.05)

Collection date	Station	CORE		EDGE	
	Time	Dawn	Noon	Dawn	Noon
17 Oct. 2002		1.51 ± 0.18a	1.81 ± 0.17a	0.18 ± 0.08d	0.30 ± 0.12d
18 Oct. 2002		2.30 ± 0.29b	1.76 ± 0.20a,b	0.36 ± 0.05d,e	0.44 ± 0.23e
8 Nov. 2002		0.24 ± 0.05c	0.24 ± 0.04c	0.00	0.00

Figure 4.5. Diel length-frequency distributions of red drum sampled at the core (dashed bars) and edge (open bars) over the course of 2 consecutive days. The upper portion of each graph shows dawn and the bottom noon collections. The study coincided with a strong settlement event.

Figure 4.5.


17 Oct. 2002
n=417
CORE
EDGE
DAWN
NOON
Frequency (%)
40
30
20
10
0
10
20
30
40
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Standard length (mm)

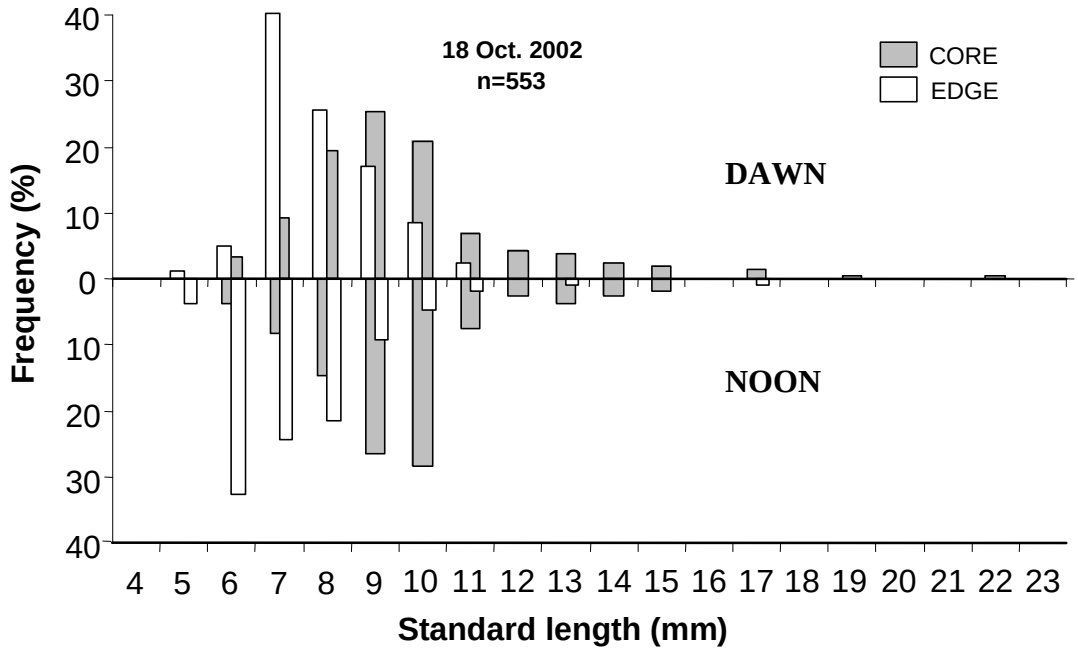

18 Oct. 2002
n=553
CORE
EDGE
DAWN
NOON
Frequency (%)
40
30
20
10
0
10
20
30
40
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Standard length (mm)

Figure 4.6. Diel length-frequency distributions of red drum sampled at the core (dashed bars) and edge (open bars). The upper portion of each graph shows dawn and the bottom noon collections. The study coincided with the post-settlement period. No fish were caught at the edge station.

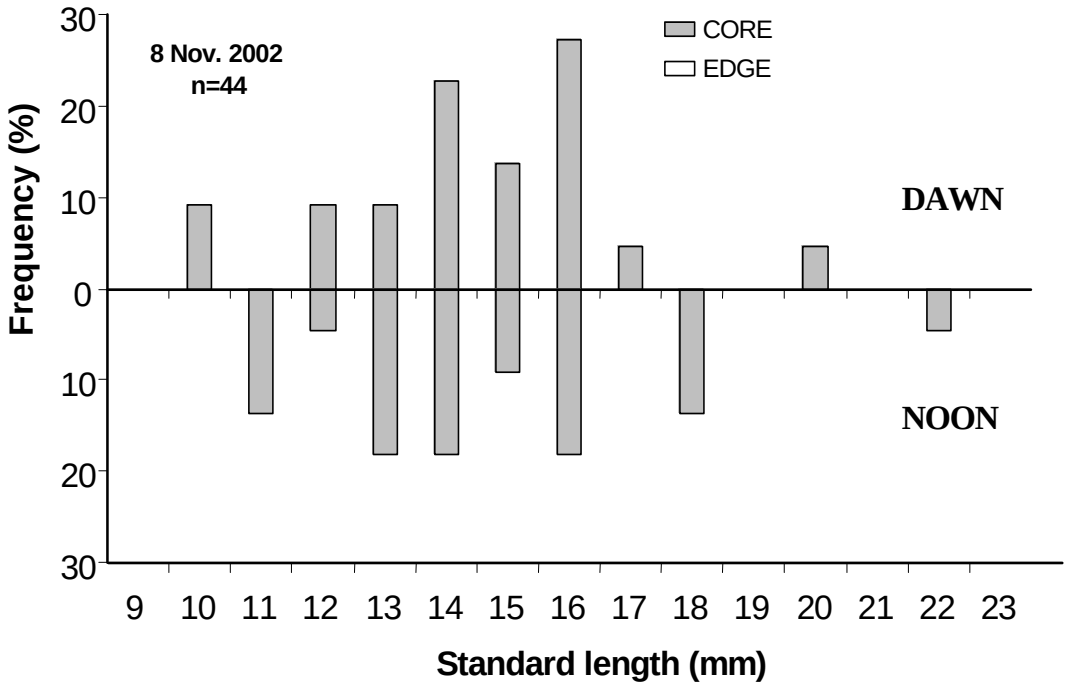
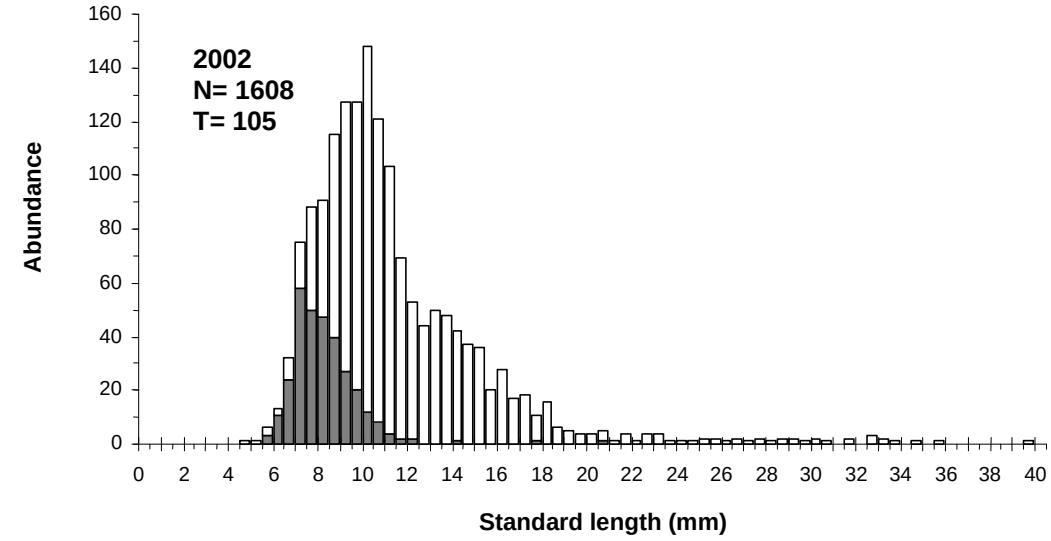
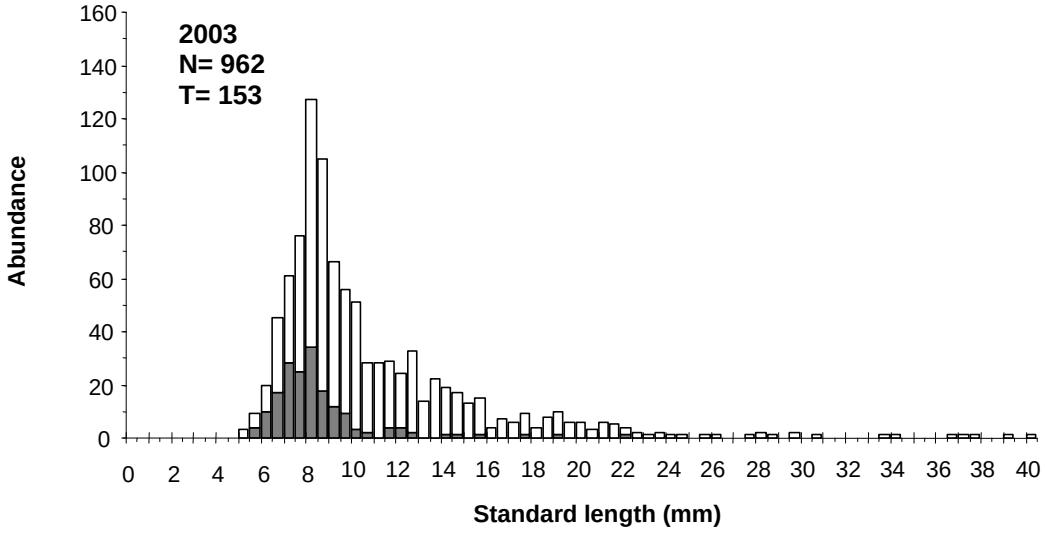


Figure 4.7. Length-frequency distribution of red drum collected in the 2002 and 2003 seagrass surveys. Shaded portion of columns represents fish caught at the EDGE station and the open portion fish caught at the CORE station. N represents the total number of fish collected and T the total number of tows conducted.





Modal Progression Analysis (MPA)

The sequence of length-frequency histograms showed distinct modes indicating the presence of fish of similar age (cohorts) (Fig. 4.8). Resolution of the length data into size modes depended on the number of observations and their similarity. Best results came from the 0.5-, and 1.0-mm class sizes. In trips with a low number of captures, the size distribution was uncertain and the sample was not used in the analysis. Cohorts were clearly distinguishable at settlement but vanished quickly (Fig. 4.8). Only the strongest cohorts were followed over several collections. Four and six distinct cohorts were identified in 2002 and 2003, respectively (Fig. 4.9). Mean size of cohorts when they were first detected (settlement) ranged from 7.4-8.0 (2002) and 5.5-6.2mm (2003). New pulses disappeared in late-October and early-November in 2002 and 2003, respectively (Fig. 4.8). Nearly flat lines represented the last collections in both years, indicating increased variability in the sample and low number of captures. All new cohorts entering the study area were detected both in EDGE and CORE surveys, although the relative contribution to the sample was much greater in the EDGE station.

Growth

Mean instantaneous growth coefficients in length (G) were 0.0020 DD_{eff}^{-1} (0.029 d^{-1}, expressed relative to days) and 0.0017 DD_{eff}^{-1} (0.026 d^{-1}) in 2002 and 2003, respectively. This inter-annual difference was observed in the steeper slope of the growth regressions in 2002 compared to 2003 (Fig. 4.9). Intra-annual variability was restricted to the year 2002, in which growth of the oldest cohort identified (A) was significantly reduced compared to the other two cohorts for which growth estimates could be made.

Figure 4.8. Sequence of smoothed length-frequency histograms by sampling day for the 2002 and 2003 red drum collections, showing the progression of dominant modes during the settlement season.

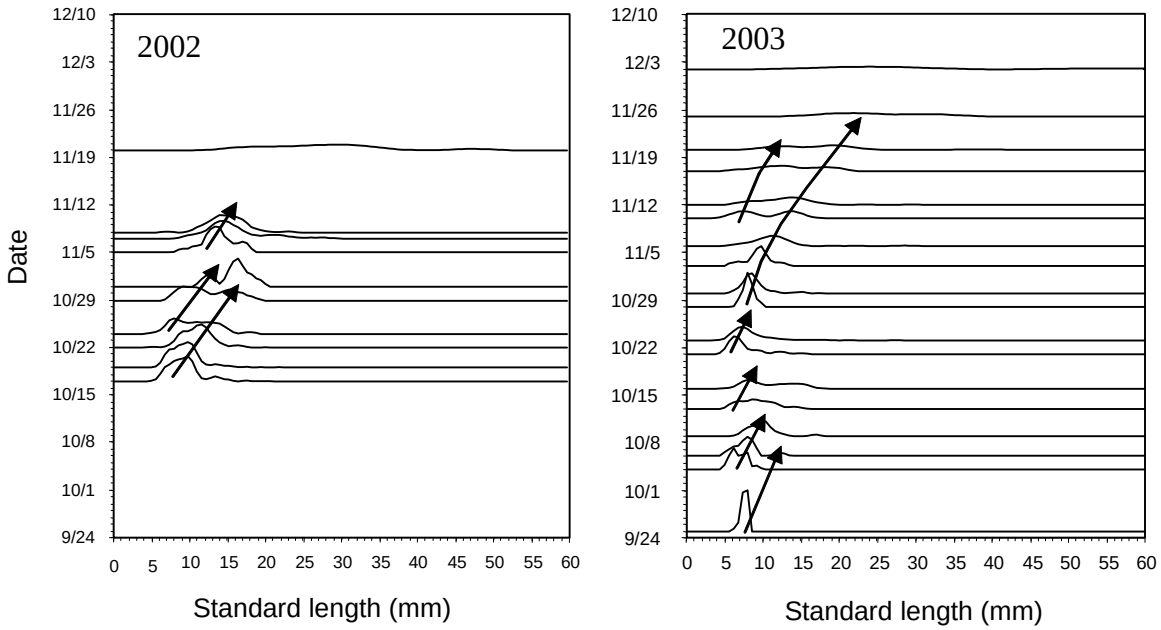


Figure 4.9. (A) Sequence of Gaussian components identified in the modal progression analysis (means ± S.D.) for the 2002 and 2003 settlement seasons. Lines represent exponential growth trajectories for means assigned to the same cohort. Diamonds represent collection dates. Numbers next to the diamonds indicate total number of individuals in the collection. Collections indicated with open diamonds were not used in the analysis. (A) age expressed in effective degree-days (DD_{eff}) and (B) age expressed in days.

Figure 4.9.

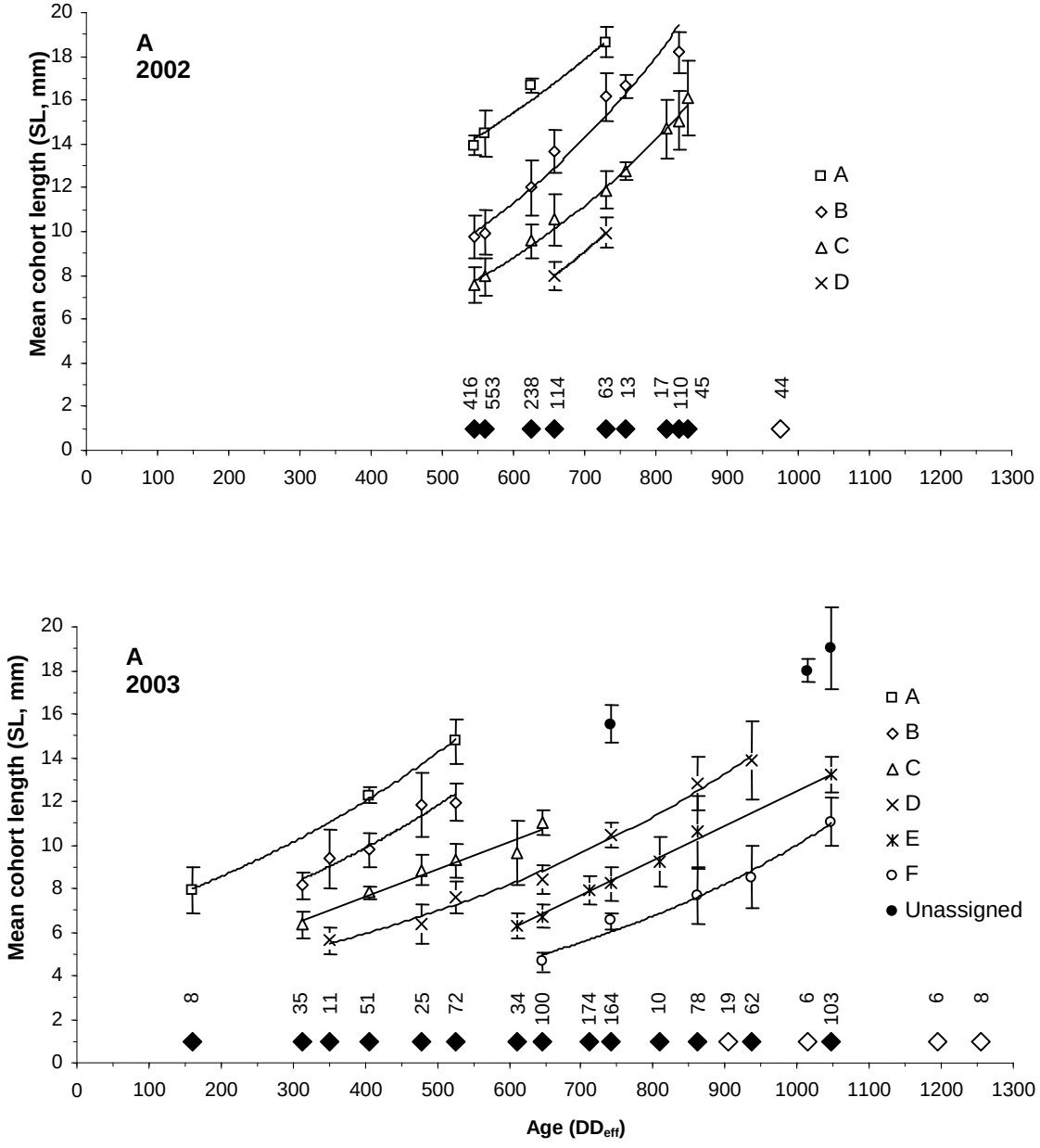
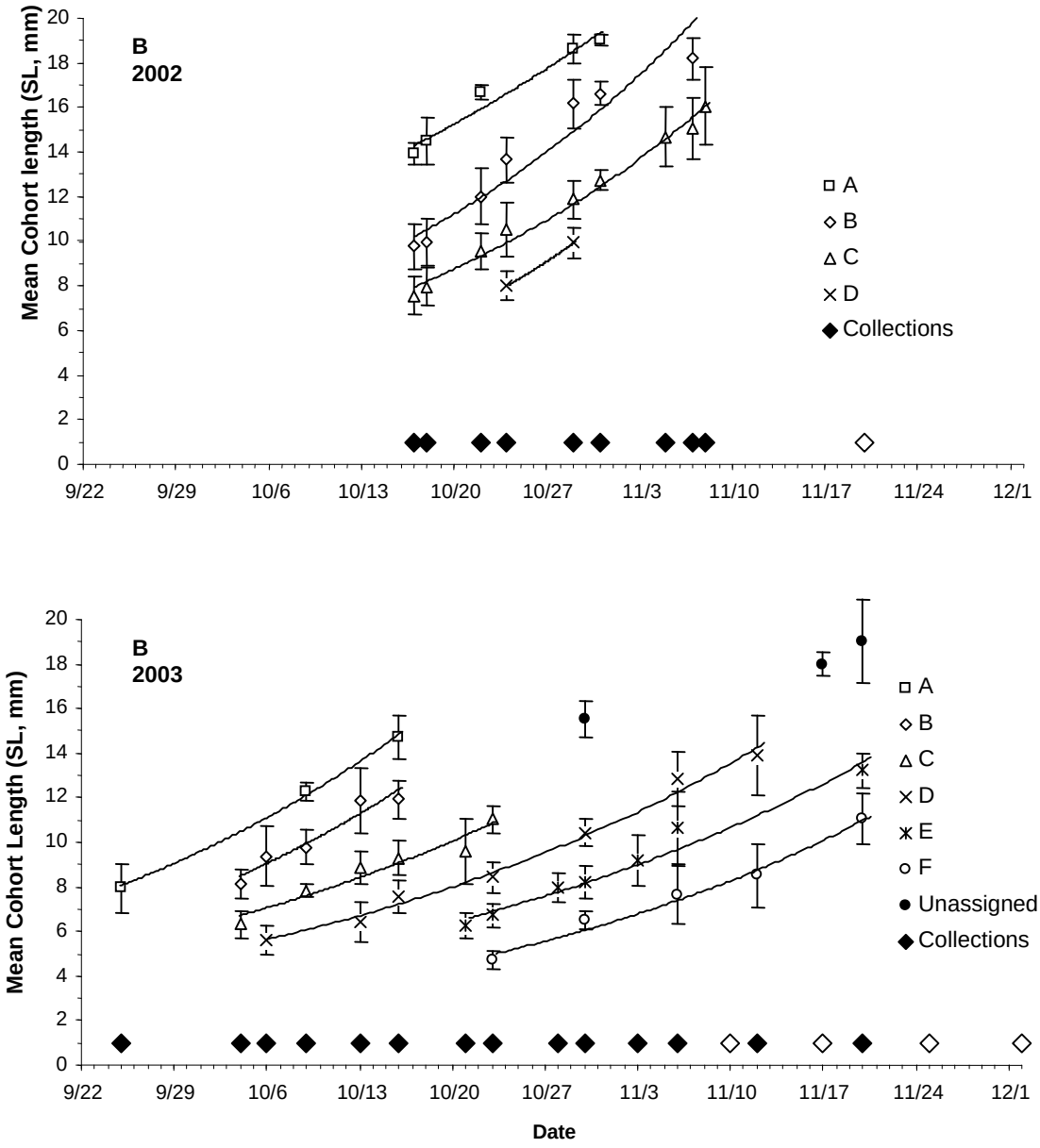

A
2002
Mean cohort length (SL, mm)
A
B
C
D
416
553
238
114
63
13
17
110
45
44
A
2003
Mean cohort length (SL, mm)
A
B
C
D
E
F
Unassigned
8
35
11
51
25
72
34
100
174
164
10
78
19
62
6
103
6
8
Age (DDeff)

Figure 4.9.


B
2002
Mean Cohort length (SL, mm)
0
2
4
6
8
10
12
14
16
18
20
9/22
9/29
10/6
10/13
10/20
10/27
11/3
11/10
11/17
11/24
12/1
A
B
C
D
Collections
B
2003
Mean Cohort Length (SL, mm)
A
B
C
D
E
F
Unassigned
Collections
Date

Mortality

Catch curves used to estimate the apparent mortality (Z) of each cohort were calculated from the cohort-specific abundance data generated in the MPA (Fig 4.10). All regression slopes (Fig. 4.11, and 4.12) were significantly different from zero except for the last cohort (F) of 2003, consequently no mortality estimate could be calculated for this cohort. Mortality estimates were more variable and imprecise than estimates of G. Due to this variability no inter-annual differences in mortality were detected. Annual mortality estimate was roughly two-fold greater in 2002 (0.0107 DD_{eff}^{-1}) compared to that of 2003 (0.0065 DD_{eff}^{-1}) (Fig. 4.13).

G':Z ratios

G':Z estimates mirrored those of the mortality estimates (Fig. 4.13). They were variable with no noticeable pattern. All cohorts but one (B, 2002) had their 95% confidence intervals crossing the threshold for positive biomass increases (G':Z=1). The mean annual estimate of G':Z was less than 1 in 2002 and greater than 1 in 2003.

Cage experiments

Due to partial or total cage loss at the EDGE station, it was only possible to compare growth and mortality between EDGE and CORE stations in one of the three deployments conducted in 2002. Corrective measures were implemented in 2003 yet only three out of four experiments could be completed. During recovery of caged fish, a relatively large number of small sized gobies and crustaceans (snapping shrimps, [*Alpheus* sp.], hermit crabs [*Paguristes hewatti*], and small blue crabs [*Callinectes sapidus*] were found in the enclosure along with red drum larvae. On only one occasion throughout the study a 35-mm juvenile seatrout (*Cynoscion nebulosus*) was retrieved along with a solitary red drum larva. This cage was not included in the analysis.

Figure 4.10. Mean density of red drum larvae per collection in the CORE seagrass during the 2002 and 2003 settlement season. Values are represented as the contribution of each cohort to the total abundance.

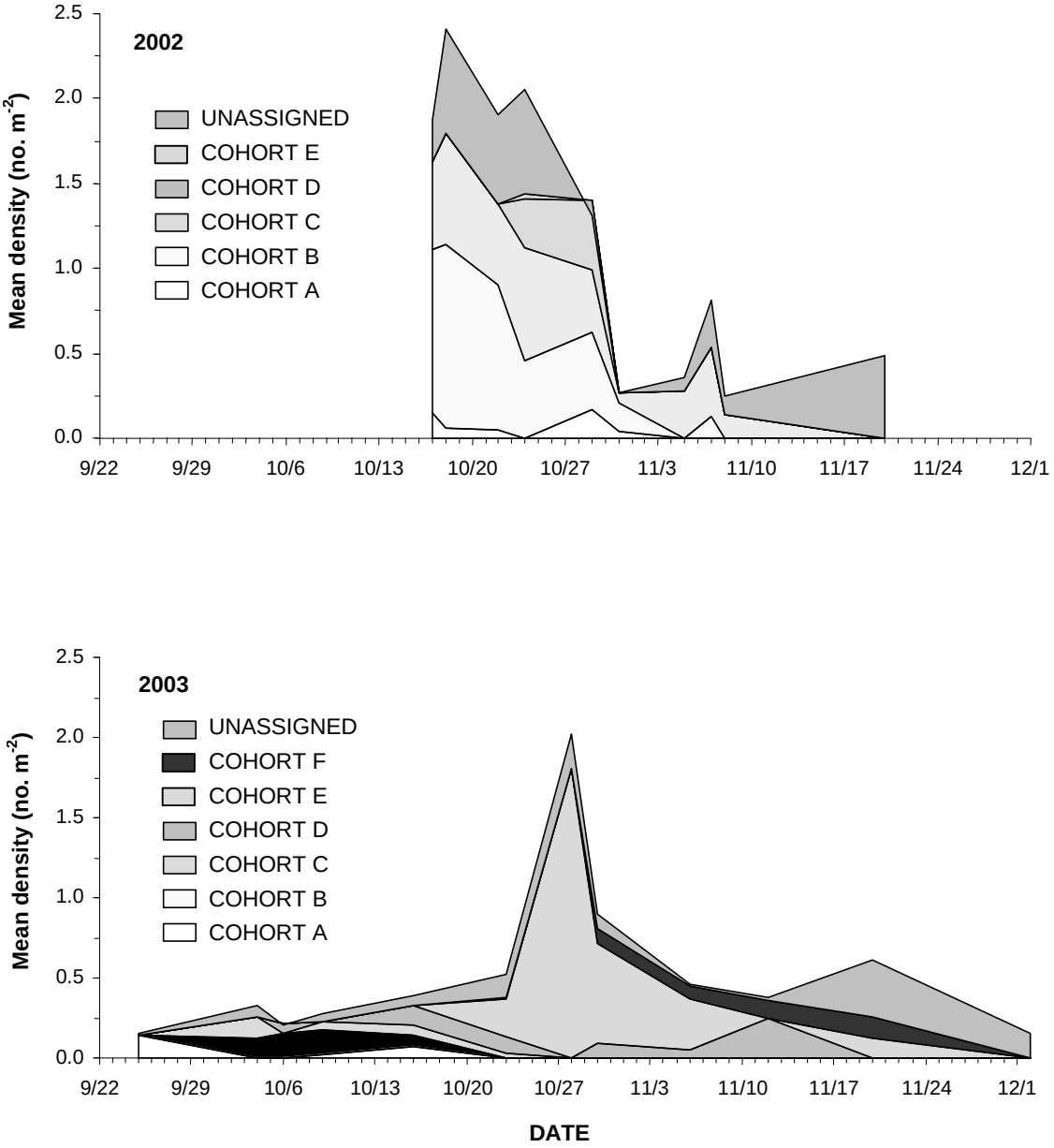


Figure 4.11. Cohort-specific Ln abundances (N_t) of settled red drum during the 2002 settlement season.

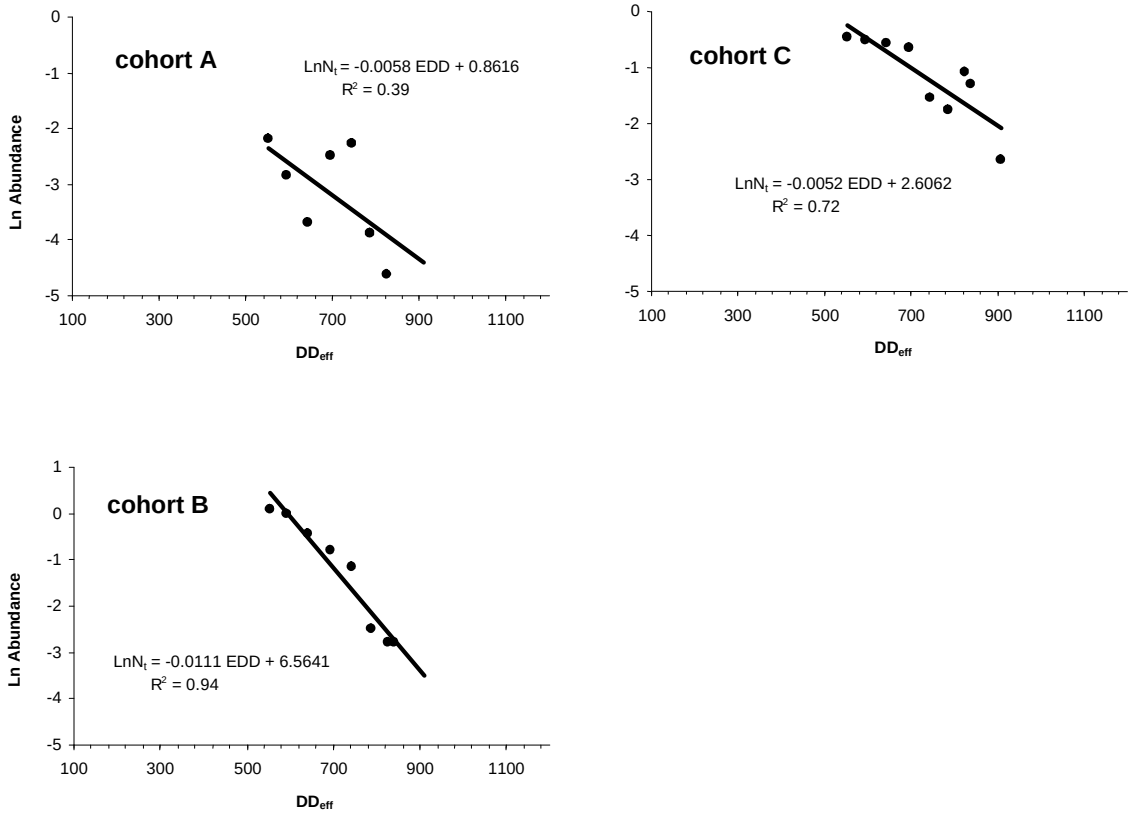


Figure 4.12. Cohort-specific Ln abundances of settled red drum during the 2003 settlement season. Triangles indicate abundance data excluded from the regressions due to incomplete recruitment.

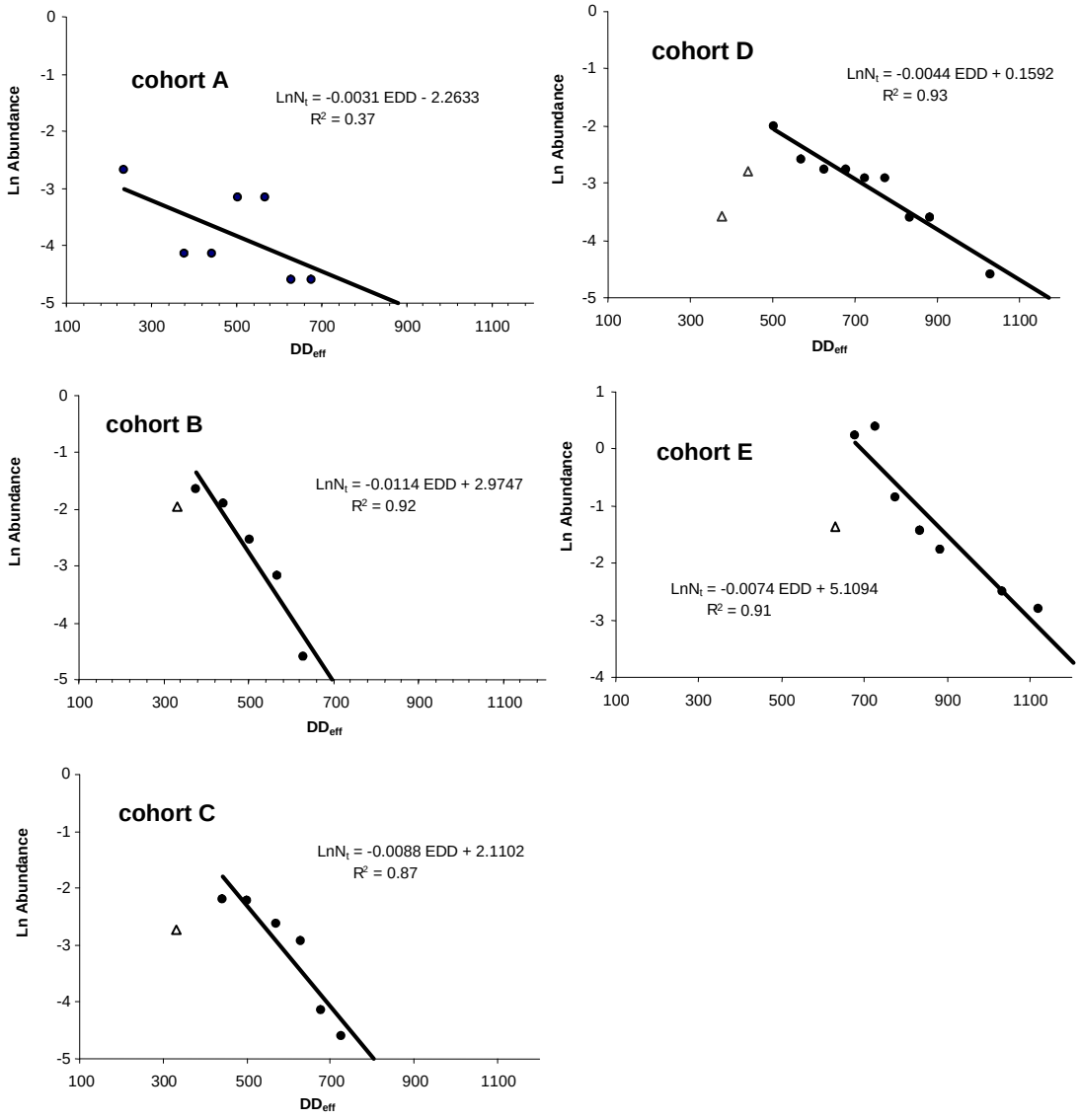


Figure 4.13. Cohort-specific instantaneous coefficient of growth in weight (G', open bars), mortality (dashed bars, Z), and recruitment potential (G':Z, bottom graph) of settled red drum collected in the 2002 and 2003 settlement season. Estimates for the overall population are shown to the right of the vertical dotted line. Error bars indicate the 95% confidence interval for the estimated parameter. The letters indicate the different cohorts. (A) age expressed in effective degree-days (DD_{eff}) and (B) age expressed in days.

Figure 4.13.

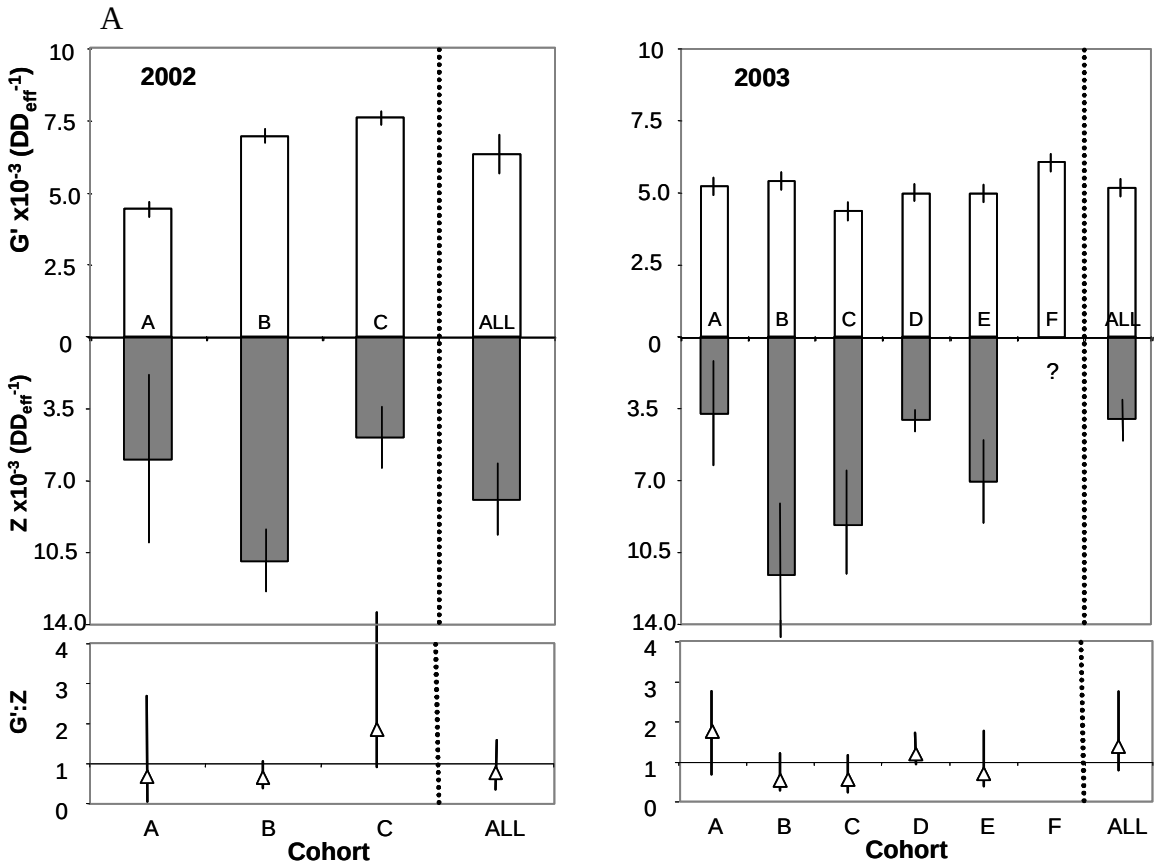
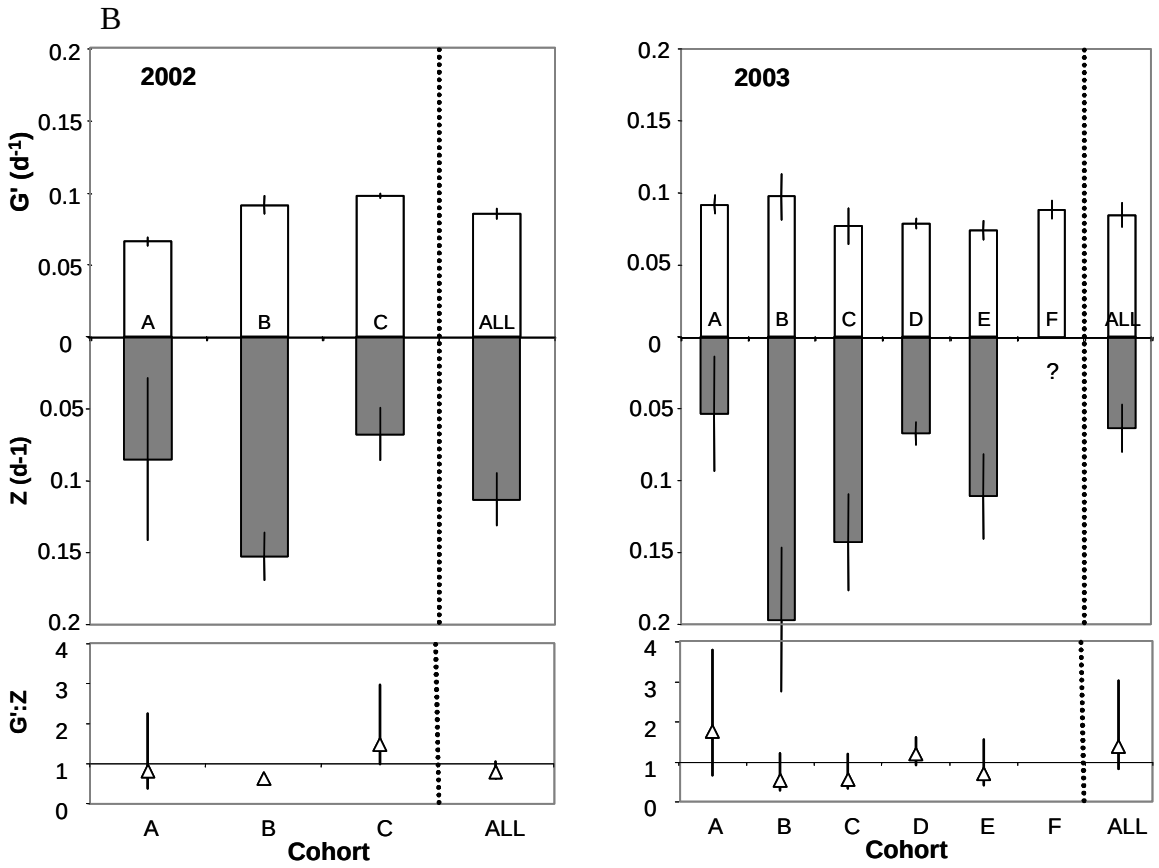

A
2002
2003
G' x10-3 (DDeff-1)
Z x10-3 (DDeff-1)
G':Z
10
7.5
5.0
2.5
0
3.5
7.0
10.5
14.0
4
3
2
1
0
A
B
C
D
E
F
ALL
?
Cohort

Figure 4.13.



Caged fish performance in EDGE and CORE seagrass

Overall growth rates were not different between EDGE and CORE stocked fish and these were generally significantly lower than that of the control fish (CTRL) (Fig. 4.14). In the third caging experiment of 2002 (C2002-3) (Fig. 4.1), fish stocked at the EDGE did not grow over the week-long deployment and showed poor condition with respect to fish stocked at the CORE or CTRL. Ambient salinity during the beginning of this deployment was extremely low and showed a greater range of fluctuation at the EDGE station (Table 4.3). Mortality was generally not different between EDGE and CORE stocked fish (Fig. 4.14), except for C2003-3, where there was significantly higher mortality in fish stocked at the CORE compared to EDGE and CTRL fish, where practically no mortality was observed.

Caged fish performance compared to wild fish

Overall, caged fish showed similar growth rates as those estimated for natural cohorts (Fig. 4.15) indicating that caging experiments may provide a useful tool to estimate growth rates of red drum settlers. Mortality was not statistically different between caged and wild fish (Fig. 4.15) probably due to the intrinsically high variability in the mortality estimates. In C2002-1, however, mortality rates estimated for wild fish (from cohorts A and B) were 4-fold greater than that experienced by caged fish, indicating that predation was probably an important component driving losses in the wild population. This deployment was the only caging experiment that coincided with relatively high density of wild settlers during the 2-year study.

Figure 4.14. Instantaneous coefficient of growth in length (G, open bars), and mortality coefficients (Z, dashed bars) (means ± SE) of caged red drum at the EDGE and CORE stations compared to control fish held in the laboratory, with food (CTRL) or under starved conditions (sCTRL). N= total number of cages or tanks used per treatment. na. not available. Bars sharing same letters (growth) or numbers (mortality) were not significantly different (P>0.05).

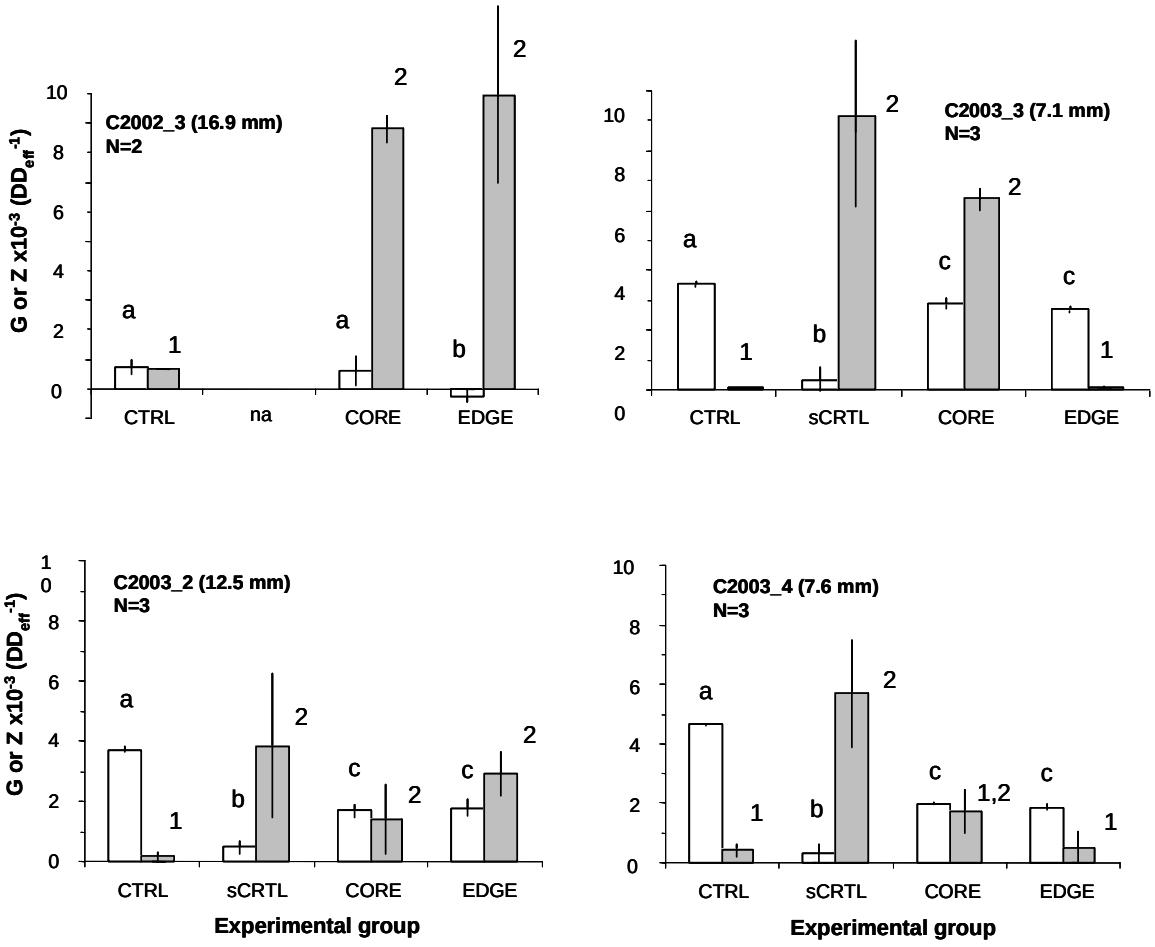
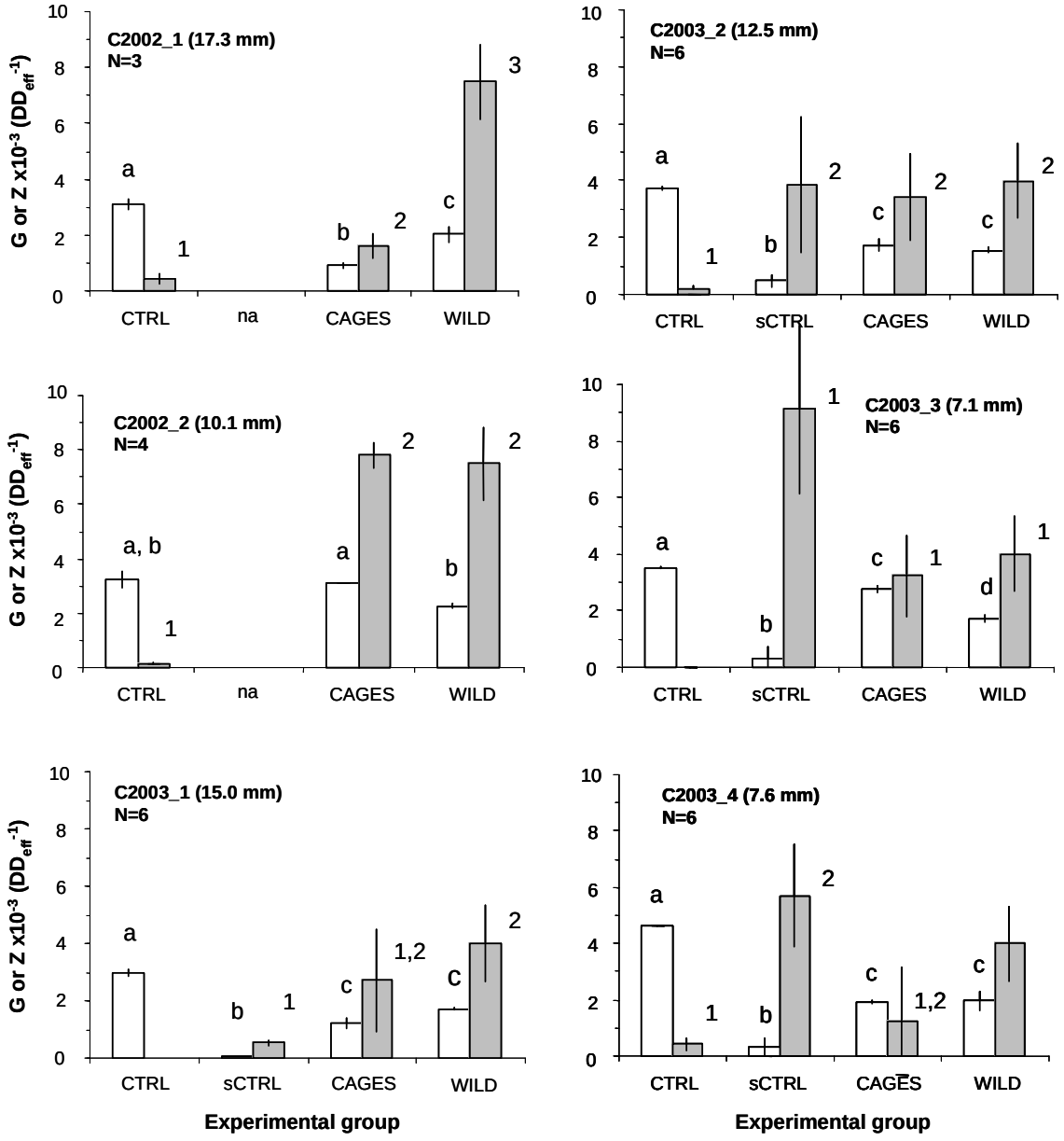


Table 4.3. Environmental conditions during caging experiments. N_0 indicates the number of fish stocked in the cages or laboratory control tanks. Number in parentheses indicates the initial size of red drum. Laboratory controls, CTRL and sCTRL, were maintained at similar conditions, only CTRL is shown.

Cage experiment (SL, mm)	Experimental interval			Temperature (ºC)			Salinity (psu)			Dissolved oxygen (mg O_2 l^{-1})			
	N_0	DD_{eff}	days	mean	max	min	mean	max	min	mean	max	min	hypoxia* %
C2002.1 (17.3)													
Edge	30	112	7	24.7	30.5	19.2	19.1	27.3	14.3	8.4	18.1	4.2	3.0
Core	30	109	7	24.4	31.3	18.5	18.2	26.0	14.2	7.0	14.2	2.1	31.6
CTRL	60	141	7	26.6	27.0	25.5	26.3	27.2	25.3	6.2	6.7	5.9	0
C2002.2 (10.1)													
Edge	50	LOST		19.7	26.0	13.8	7.8	25.8	2.2	9.9	19.5	6.04	0
Core	50	118	11	19.5	27.4	12.1	7.5	18.1	3.1	8.2	18.5	4.1	4.8
CTRL	100	127	11	19.8	23.6	17.5	7.0	9.9	5.5	8.5	9.2	7.4	0
C2002.3 (16.9)													
Edge	30	68	7	19.7	24.0	15.2	15.9	27.6	3.1	9.0	15.3	3.5	5.3
Core	30	68	7	19.9	27.0	13.6	14.2	24.3	6.9	7.0	14.7	1.7	27.3
CTRL	60	74	7	19.3	21.4	17.1	14.0	15.4	12.3	8.1	8.5	8.0	0
C2003.1 (15.0)													
Edge	12	140	8	26.3	30.9	23.8	24.2	30.1	18.6	7.0	13.8	1.9	7.7
Core	12	142	8	26.4	31.8	23.2	23.9	29.7	5.72	6.3	11.15	1.44	27.3
CTRL	12	166	8	26.3	26.7	25.9	25.3	-	-	-	-	>5.0	0
C2003.2 (12.5)													
Edge	14	173	10	25.9	29.9	20.8	21.8	26.7	17.9	7.0	13.1	2.3	12.4
Core	14	173	10	25.9	30.9	20.8	22.1	25.8	19.3	6.1	11.0	2.0	33.8
CTRL	14	161	10	24.4	26.6	23.6	22.4	-	-	-	-	>5.0	0
C2003.3 (7.1)													
Edge	25	159	11	23.5	29.4	16.7	30.8	33.0	27.7	6.6	14.8	2.4	29.2
Core	25	162	11	23.8	30.8	16.7	29.8	33.0	22.8	6.5	13.4	1.6	36.3
CTRL	25	187	11	25.8	26.7	23.9	30.1	-	-	-	-	>5.0	0
C2003.4 (7.6)													
Edge	12	143	9	23.6	29.2	14.9	29.3	31.3	27.8	6.7	8.9	5.2	0
Core	12	141	9	23.3	29.2	12.4	31.7	35.4	24.0	6.9	16.1	1.8	30.6
CTRL	12	140	9	23.9	24.7	22.85	29.3	-	-	-	-	>5.0	0

* Total time spent at DO concentrations below the computed LOC

Figure 4.15. Instantaneous coefficient of growth in length (G, open bars) and mortality coefficients (Z, dashed bars) (means ± SE) of control (laboratory) and caged red drum compared to field estimates derived from fish surveys. Labels are same as in Fig. 4.14.



DISCUSSION

Red drum larvae settle into a very dynamic environment in which diel, seasonal and annual variations are important. Generally, conditions during September and October were comparable between years and similar to those conditions found by Rooker *et al.* (1998b and 1999) in the same area in 1994 and 1995. It is during this period of relatively high predictability that red drum initially settle, as suggested by the timing of the appearance of new cohorts. Late-season conditions were less predictable and showed substantial variability between years, including extraordinary events that may have resulted in mortality, as suggested by the sudden drop in population abundance during the very low salinity period experienced in 2002. Catastrophic environmental events associated with sudden declines in abundance are relatively frequent in freshwater and some estuarine systems in temperate areas (Houde 2002). Rutherford and Houde (1995) reported significant mortality in larval cohorts of striped bass (*Morone saxatilis*) coincident with the passage of strong cold fronts. Red drum could be at relatively higher risk of recruitment failure due to these weather-related events than other fish species inhabiting more stable but less productive habitats. Density-independent mortality due to stochastic weather patterns will add another level of uncertainty to the recruitment process in red drum that, in some cases, may be substantial and perhaps occasionally as important as mortality determined by density-dependency, such as food availability or predation. Nevertheless, the risk of extensive mortalities due to weather-related density-independent factors will operate on postsettlement individuals rather than settling larvae since these events are likely to occur late in the season after annual settlement is complete.

Distribution patterns and connectivity of microhabitats

Previous surveys of the study area (see chapter 2) determined the presence of two contrasting microhabitats within a large seagrass meadow: the core seagrass and the edge seagrass (defined as the deeper boundary of the seagrass meadow). Core seagrass areas experience transient hypoxia events at dawn that are more severe than at the edges. Changes in fish distribution patterns among different microhabitats can be important in determining growth and mortality rates (Carr and Hixon 1995; Planes and Lecaillon 2001; Srinivasan 2003). There is also evidence that some organisms have the ability to position themselves in particular locations within habitats (Atema, *et al.* 2002; Stoner and Titgen 2003).

I hypothesized that diel differences in microhabitat characteristics were strong enough to induce directed movements of red drum. This hypothesis was tested by collecting larvae at the extremes of the range in environmental condition timed with the phase in which the change occurs. Diel DO changes found during the sampling dates were not among the more extreme environmental fluctuations documented at the study site. DO only dropped at the CORE station to 61-68% saturation during the first two collections, and to 72% saturation during the last collection date. Diel temperature changes were within the normal 3-6 ºC range typically found in these habitats. Under these conditions, there were no significant differences in abundance or length-frequency distribution between dawn and noon samples at any combination of location and date, indicating that red drum do not exhibit diel movements between core and edge seagrass habitats.

Spatial and temporal variation in habitat characteristics are probably the most studied patterns in nature that direct distribution of natural populations. However, in the context of recruitment of early life stages to their nursery grounds, advection pathways

and settlement patterns may determine the actual dynamics of colonization and initial habitat use. The fine scale temporal and spatial mechanism of red drum settlement apparently involves differential use of two areas of the seagrass. Length-frequency distributions of edge and core samples showed that smaller individuals are more likely to be found at the edge of the seagrass and that bigger individuals (>12 mm SL) are normally in core samples, suggesting that deeper seagrass areas bordering the meadows function as a transitional habitat during the initial phases of settlement and the core acts as the main nursery habitat where recruits accumulate. Whether or not this is an accurate depiction of the actual settlement process is arguable since settlement is not measured directly, but estimated from abundance of presumed new individuals (smaller fish) sometime after the actual settlement event. The alternate hypothesis of size-selective mortality does not explain the differences observed in the abundance and size distribution patterns as well. The diel experiments suggest that red drum reside permanently within the core of the seagrass once settlement is complete, as indicated by the lack of diel movements of the large, and potentially superior swimmers found late in the season and their absence from edge seagrass areas.

The two collections of the first diel experiment were coincident with the initial settlement of cohort C (2002). The observed size distributions of fish during the 2-d period of the surveys seems to favor the hypothesis of initial settlement at the edge sustained for at least 2-d (duration of the diel surveys). Cohort-specific catch curves obtained in 2003 suggest an active recruitment period for each cohort that spanned about 1 week (two consecutive collections). Fish caught at the edge seagrass were significantly smaller that those found at the core seagrass. Also abundances roughly doubled at the edge over the 2-d period, suggestive of fish accumulating in the area. The length-frequency distribution of edge-caught fish shifted up by almost one entire size class (1

mm) in less than a day, suggesting that growth was probably not entirely responsible for this switch since average growth rates reported for wild red drum larvae range from 0.26 (Rooker et al. 1998b) to 0.39 mm day^{-1} (this study). The parsimonious interpretation is that new and bigger fish are arriving and settling into the edge on the second collection day, suggesting that fish that had hatched on different dates are settling at the same time. Present evidence supports the theory of a mixed pool of potential settlers since red drum spawning activity occurs daily during the reproductive season (Holt 2003; Wilson and Nieland 1994). However, the majority of red drum appear to settle in discrete pulses (size cohorts), suggesting a major role of physical factors controlling the transport of larvae to the nursery grounds (Brown *et al.* 2000, 2004). Holt *et al.* (1989) examined the passing of presettlement red drums through the inlet connecting the Aransas Estuary with the spawning grounds and found directed vertical movements of red drum larvae cued to tidal and diel cycles, suggestive of active behavior favoring transport into the estuary. Oceanographic processes may create especially favorable conditions for transport that, coupled with behavioral responses of the larvae, are responsible for the formation of the size cohorts. Evidence from other species suggest that settlement is often characterized by brief pulses of intense settlement between periods of no or relatively small input (Doherty and Sale 1986; Danilowicz 1997; Schmitt and Holbrook 1999). Herzka *et al.* (2002) presented a contrasting observation in which new settlers were detected on a daily basis, suggestive of gradual colonization of the nursery grounds. Without further studies formation of the cohorts cannot be explained. However, this study shows that there are groups of fish of similar size settling at the same time and that their progression in size can be followed during the early postsettlement period.

What is the role if any of the deep seagrass areas? Herzka *et al.* (2002), using stable isotopes as a measure of the relative time since settlement was able to estimate the

size at settlement of post-settlement, red drum caught at core seagrass sites. They reported a wide range of sizes for new settlers that was mainly centered on the 6- 8-mm size classes. In this study, these size classes settled at the edge sites. These size classes were within the ascending limb of the catch curve calculated from core collections (Rooker *et al.* 1999, and this study) and consequently not yet fully present in the core habitat. This supports the idea that settlement actually occurs at the edge and therefore credits edge seagrass as essential habitat for red drum settlement. Core samples did include some of the smallest individuals caught, although in relatively low numbers, indicating that red drum are able to settle directly to the core of the seagrass. There is some evidence suggesting that mortality at, and immediately after, settlement can be substantial, ranging from 10% to as much as 90%, and it is usually size-dependent, with highest losses for the smallest individuals (Doherty and Sale 1986; Tanaka *et al.* 1989; Carr and Hixon 1995; Planes and Lecaillon 2001). Those studies were conducted on coral reef fishes and flounders, groups for which most settlement information is available, and suggest the existence of a window of high mortality that can influence recruitment variability (Blaxter 1988; Searcy and Sponaugle 2001). If red drum follow these general patterns and experience high mortality associated with settlement, deep seagrass surveys must be included in studies to understand the dynamics of recruitment in the species, in particular the importance of larval supply relative to later density-dependent processes regulating recruitment.

In 2002, the length-frequency distribution of red drum collected at the edge seagrass was shifted towards smaller size classes compared to core seagrass. However, total catch in 2003 for both core and edge surveys was centered at approximately the 8-mm SL size class, similar to data reported by Rooker *et al.* (1999). Two factors may have contributed to the shift observed in 2002: first, disproportionate contribution to the total

catch (60.3%) of the first two surveys conducted when a cohort was settling, and second, the fact that the surveys started when two cohorts had settled and moved to the core seagrass area and consequently their smaller size classes were missing from the catch curve. These results underscore the importance of a comprehensive sampling strategy covering the entire settlement season and both strata (edge and core) to draw meaningful conclusions about recruitment from length-frequency curves in red drum.

Caged fish performance in CORE and EDGE seagrass

Detailed analysis of growth and mortality in relation to fine scale habitat properties are often difficult to do with the necessary precision and reasonable effort from field collections (Planes and Lecaillon 2001). In chapter 2 we determined, under controlled laboratory conditions, that different temperature and dissolved oxygen regimes characteristic of the nursery have no measurable impacts on growth or mortality. Cages were intended to explore responses to natural conditions in the nursery that cannot be easily simulated in the laboratory. The penalty of doing so is much less control over the experiment, and consequently the results must be interpreted with caution.

Edge- and core-caged fish showed similar growth rates, suggesting that both areas of the seagrass were similar in terms of habitat characteristics that control growth rate (food quantity and quality, and environmental condition). These results confirm our previous laboratory results that suggested no effects of diel environmental differences. However, caged fish had generally lower growth rate compared to fed controls but always outperformed starved controls, suggesting some sort of food limitation in the cages. Temporal variability in food abundance is common in local estuarine systems (Montagna and Kalke 1992) and this may have accounted for some the differences.

Mortality in fed controls was almost negligible, demonstrating that red drum larvae readily tolerate the stresses associated with handling and transportation. Mortality

estimates were in general similar between edge and core seagrass. Caged fish had greater mortality than fed controls but lower than starved controls. Interestingly, in just one experiment (C2003-3) mortality was at the control level in edge cages (93.5 ± 2.5%, mean survival ± SE) and significantly lower than the core counterparts (P <0.001). This is an isolated observation that needs further confirmation before definitive conclusions can be made, however the same trend, although not statistically significant, was observed in C2003-4. Both caging experiments were stocked with small red drum larvae (7.1 and 7.6 mm SL), a size that corresponds with initial settlement at the edge seagrass (suggested by the field collections). This may indicate that edge seagrass is a superior habitat for settlement than core seagrass. Further investigations are needed to clarify these observations.

Growth and mortality of natural cohorts

Modal Progression Analysis (MPA) adopted in this study provides an alternative method to estimate vital rates of red drum cohorts in nursery grounds. This approach has not been commonly used in larval fish studies despite its relatively wide application for later stages of fishes. However, under certain circumstances MPA can be a very useful method for population estimates of growth and mortality. Scales of time and size are very different. For larvae, time is measured in days or DD_{eff} rather than years and size in tenths of millimeters instead of centimeters. But this scaling does not seem to affect the validity of the results (Ebert *et al.* 1993). Cohorts were defined in this study by their size rather than age, although due to the relatively young age at settlement and the relative uniformity of the pre-settlement pelagic environment, these two metrics must be closely related. Nevertheless, Fuiman *et al.* (1998) proposed size as a superior estimator of ontogeny than age in larval fish since it has little variability and potentially integrates environmental experience of the fish (food availability, temperature, etc.). On the other

hand, MPA estimations of growth during the time in which the cohort is actually settling may potentially introduce bias and underestimate the true growth rate of the population since the addition of new and smaller recruits will prevent the population mean from accurately tracing the growth of individuals (Sanvicente-Añorve *et al.* 2003). Similar concerns can be raised in the presence of size-selective mortality (Gleason and Bengtson 1996), However, confounding effects due to movements are the most important problem of the MPA (Sparre *et al.* 1989; Fournier *et al.* 1998). The main difficulty encountered in this analysis was the overall low density of fish compared with other surveys conducted in the same area (Rooker *et al.* 1999). By the end of the sampling period, the combined effects of low catches and large variability in the sizes of the fish making up the collection made it impossible to conduct any analysis. At the densities and effort level used in the study, the upper size range that could be analyzed was 16-20 mm, which corresponded to a period of about 2 to 5 weeks postsettlement. This is a relatively narrow window but most of the mortality affecting the settlement of fishes may occur within the first week following their initial detection within the nursery area (Doherty and Sale, 1986; Tanaka *et al.* 1989; Carr and Hixon, 1995; Planes and Lecaillon 2001).

In our analysis, length-specific growth rates averaged 0.0020and 0.0017 DD_{eff}^{-1} (0.39 and 0.26 mm day^{-1}) in 2002 and 2003, respectively. This is substantially lower than those reported by Rooker *et al.* (1999) which were 0.58 to 0.62 mm d^{-1}. The different approaches to estimating growth between the studies could be partially responsible. MPA follows subsets of the population over time and consequently provides population estimates. Individual-based processes are integrated into the averaged set of values that define the population. This work aimed to derive insight into the recruitment potential of red drum larvae as they use the nursery grounds. Recruitment is a population attribute, therefore an estimate of the population (or in this case cohort) growth rates based on the

population itself could better approximate any index of recruitment we use, given sufficient number of observations.

In any case, growth rates will depend on provision of food and environmental conditions and all of these factors can vary between years. Temperature was probably not responsible for the small differences in growth rate observed between the 2 years of this study because our growth estimates are based on effective degree-days and therefore largely independent of temperature since during both years temperatures were within the range in which the growth response of red drum to temperatures is fairly linear (Fry 1971; Kamler 1992). Rooker *et al.* (1999) reported greater growth in mid-season cohorts and speculated that growth was not related to temperature but to other characteristics of the environment that they could not identify. No common pattern in growth rate estimates was observed across the two seasons, suggesting that growth conditions were similar throughout the period in which the cohorts in the nursery were followed.

Cohort-specific mortality rate estimates averaged 10.7% d^{-1} and 11.7% d^{-1} in 2002 and 2003, respectively and were comparable to those reported previously (12.5-13.0% d^{-1}, Rooker *et al.* 1999). Rooker *et al.* (1999) reported lower mortality in cohorts spawned in the middle of the reproductive season. In the present study, cohort-specific losses were variable and ranged from 5.5 to 21.8% d^{-1}. The variability associated with the growth-rate estimates was low (mean R^2 >0.95), however mortality-rate estimates contain a substantial amount of variability (mean R^2 = 0.71). Mortality rates derived from abundance estimates are fundamentally gross estimates and have an inherent inaccuracy (Houde 2002). The low densities encountered during most of the collections in this study further affected the ability to estimate mortality. Consequently, no trend in mortality could be found among cohorts. In addition, mortality estimates were derived from changes in abundance-at-age over time. This method has the fundamental problem that

fish movements in and out of the sampling domain cannot be distinguished from losses due to mortality. Alternative approaches using marking techniques are a formidable task due to the high mortality rates of these organisms (Jones *et al.* 1999, Jones 2002). Field estimates of mortality rates are consequently very difficult to obtain and are comparatively much less accurate than growth rate estimates (Houde 2002), posing a problem for accurately evaluating nursery habitat quality.

G':Z ratios

My approach to habitat quality was based on the relative importance of growth to losses from the population and uses the ratio between cohort-specific growth rate in weight (G') and mortality rate (Z) as an index of recruitment (Houde and Zastrow 1993; Rutherford and Houde 1995; Rooker *et al.* 1999; Houde 2002). Using G':Z as an index of recruitment, better habitat will result in greater values of G':Z since better habitats will enhance growth and survival. In both years of the study cohort-specific G':Z values were greatly influenced by mortality estimates, suggesting that mortality rates rather than growth rates were determining the potential to recruit. However, the imprecision of mortality estimates translates into a wide confidence interval that precludes detailed comparisons between cohorts and environmental conditions experienced in the nursery. In all but one case, the 95% confidence intervals for cohort-specific G':Z included 1 (G' = Z) suggesting that most cohorts could have contributed to the final recruitment in both years.

Mortality-dominated (G' < Z) processes early in the ontogeny change to growth–dominated (G' > Z) processes later in life (Houde 1987). The precise timing of this transition differs among species and will depend on habitat characteristics (Werner and Hall 1988; Werner 2002). Starting with the same number or weight of individuals, a cohort that crosses the G' = Z threshold earlier in ontogeny will contribute greater

numbers of recruits or biomass than cohorts that reach this threshold later in life. Better field estimates of mortality are needed to determine this critical size when G' = Z for red drum and to judge the importance of various factors on recruitment output.

In the nursery, disappearance of recruits can be the result of, mortality and/or movement. Therefore migration is a confounding effect inherent in most mortality estimation methods (Sparre *et al.* 1989; Planes and Lecaillon 2001; Houde 2002). This study used hatchery-reared fish to explore the use of controlled caging experiments to derive estimates of responses that wild red drum may exhibit in the field. Cages can be used to control migration and, depending on their design, predation. Concerns have been raised over the frequent occurrence of caging artifacts (Kennelly 1991; Connell 1997). Also, the use of naive fish adds problems. These concerns may limit their reliability, however knowing their limitations, cage experiments can be useful for some experimental questions (Planes and Lecaillon 2001). Recognizing that direct comparison of growth and mortality rates in cages with the rates that would occur naturally is problematic, this discussion deals with trends rather than absolute values.

Six caging experiments over the 2-year period coincided in time with the presence of natural cohorts in the nursery. There was generally good agreement between estimates of growth rate from caged fish and wild fish, indicating that hatchery-reared red drum learn to hunt natural prey quickly. Compared to laboratory controls, caged and wild fish grew at rates close to, or less than fed control but definitively better than starved controls, suggesting some level of food limitation in the field. On the other hand, Rooker *et al.* (1997), using RNA:DNA as an index of condition, found no signs of starved red drum larvae and concluded that red drum larvae were well fed and prey limitation was unlikely. I found no evidence of starved larvae in the wild but food availability may be less than

that provided to control tanks in the laboratory. On the other hand, food may be abundant but growth efficiency may be reduced under field conditions (see chapter 2).

Similar mortality estimates between caged and wild fish were unexpected. I had hypothesized that the cages would remove the effects of both movement and predation on mortality and consequently expected to have much lower mortality in the cages than in natural cohorts (as in C2002-1). The parsimonious explanation, given my assumptions are true, is either that field mortality was underestimated or that unexpected mortality occurred in the cages. The mortality estimates ($Z = 0.12\ d^{-1}$) were similar to those reported a decade earlier by Rooker *et al*. (1999) ($Z = 0.14\ d^{-1}$) for the same estuarine system, and they were within the upper range found for other marine fish larvae ($Z = 0.04\text{-}\ 0.18\ day^{-1}$) (Houde 1987), indicating that our estimate is reasonably accurate. The second explanation, an additional source of mortality in the cage, may be due to predation since only large predatory fishes and crustaceans but no benthic invertebrates were removed from the cages. Predation by benthic crustaceans has been documented in the field for plaice (*Pleuronectes platessa*) (Van der Veer and Bergman 1987), and Japanese flounder (*Paralichthys olivaceus*) (in the field Tanaka *et al*. 1989; in the laboratory, Hossain *et al.* 2002). A number of small blue crabs (< 25 mm) and snapping shrimp (<15 mm) were found consistently in the enclosures at the time of fish recovery. No information is available on the possible role of these organisms as predators of settled red drum larvae. Interestingly, the only caging trial in which mortality was reduced with respect to wild fish is the one in which the largest red drum were stocked. These observations need further confirmation but they can be interpreted as reduced predation due to larger sizes of the larvae in the cages. Another source of unaccounted mortality could be cannibalism, as suggested by laboratory studies (Chiu and Chang 2002; Kellison, *et al.* 2002). However, the densities of fish stocked in the cages ($12\text{-}50\ m^{-2)}$ were

much lower than those of the control tanks (36-300 m^{-2}), where no incidence of cannibalism or mortality effect was apparent. Reduced food availability in the cages might lead to cannibalism, especially in larger larvae, but the higher mortality was not seen in the larger caged red drum. Because of the tremendous importance of mortality in the early life history of the species, further research assessing the role of this and other benthic invertebrates is needed.

CONCLUSIONS

Results of this study suggest that the edge area within seagrass meadows is an important habitat for red drum settlement. Newly settled larvae move towards core seagrass habitats within a few days of arrival to the nursery habitat and remain there. The supply of new settlers is pulsed and probably coupled with physical transport, and it is the combination of pulsed settlement and fast growth rates that ensures a clear separation of successive size cohorts. Size cohorts arrived from early September to late October. Mortality is substantial and variable and has greater influence than growth on the value of the recruitment potential index (G':Z). No seasonal trends in G':Z were observed, but they were often greater than one (G' > Z), and probably most cohorts contributed to recruitment. Caging experiments, while exploratory, may indicate edges of seagrass beds are a superior habitat for settlement. Finally, hatchery-reared red drum stocked into field enclosures can be used to estimate growth rates of wild counterparts but fail to provide information on mortality or movement during settlement and subsequent recruitment to the nursery.

Chapter 5: Summary and Conclusions

In this study a combination of laboratory experimentation, caging studies and field collections was used, along with high resolution environmental monitoring to assess the implications of nursery environmental cycles to red drum populations during the settlement period. Fine-scale spatial settlement patterns were followed and described, and a novel sampling method developed to assess red drum use of deep-edge seagrass areas that were previously inaccessible for surveys. Endocrine development of the thyroid and interrenal (cortisol) glands was assessed, establishing and validating specific assays to measure L-thyroxine (T4) and 3-5-3'-triiodo-L-thyronine (T3), and the major shifts in hormone production were correlated with ecological transitions in the species. Size cohorts were detected as they settled into the nursery, and their progression was follow to derive growth and mortality estimates, which were combined to assess recruitment potential.

Important diel variations in DO and temperature were documented in the nursery. Hypoxia events were regularly observed especially at the core seagrass where they lasted for longer periods compared to deep-edge areas. In general, deep-edge areas were substantially more stable than core zones with respect to diel and meteorological forcing. Fronts were common late in the season and were associated with major and rapid changes in water quality parameters that may have been responsible for decreases in abundance during the study.

In the laboratory, simulated diel temperature and diel DO cycles within the range observed in the nursery did not affect growth or survival, indicating that short hypoxic events might not limit the overall growth performance of larval red drum in the nursery habitat. Red drum tolerate the normal diel variability in the seagrasses quite well and no

deleterious effects on growth or survival were anticipated under hypoxia exposures of up to 30% of the day. In the laboratory, previous exposure to environmentally-realistic temperature cycles provided an advantage to fish facing quick cooling events similar to those provoked by fronts in the nursery. To wild fish in the process of settling, this may indicate greater vulnerability to stochastic weather events compared to individuals already settled.

Relative to other marine fish larvae, red drum activate the thyroid axis before yolk absorption. This activation is probably determined by early ORD activation rather than thyroid gland differentiation. Cortisol also peaks in early larvae before yolk absorption, suggesting a contribution of the thyroid hormones and cortisol to developmental events in the early larvae. Thyroid and interrenal glands are fully differentiated before the settlement period in nature. This period coincides with elevated thyroid activity and a possible hyporesponsive period to environmental stressors. Changes during ontogeny of both endocrine axes are correlated with ecological events in the early life of red drum. This observation underscores the importance of detailed studies on the mechanisms through which these hormones control development and adjust the larvae to the nursery during settlement. No cortisol stress response is activated in response to environmentally-realistic diel cycles or hypoxia, suggesting the lack of compensatory responses to brief nocturnal hypoxia which could result in substantial energy investments.

This work demonstrates that there is an important pulsed component in the supply of new settlers to the nursery, probably coupled with physical transport. It is precisely the combination of pulsed settlement and fast growth rates that ensures a clear separation by size of the successive size cohorts and the applicability of the Modal Progression Analysis to red drum settlement dynamics. The results suggest that edge seagrass is used by smaller size classes and that it is an important habitat for red drum settlement.

However, the use of these areas is brief, and settled larvae move towards core seagrass habitats within few days of their arrival at the meadow where they spend the entire postsettlement period. There also were indications of direct settlement at the core seagrass, however, the relative importance of each habitat during settlement could not be quantified. The relative stability of edge seagrass with respect to environmental fluctuation may be beneficial during the necessary adjustment to the new benthic environment by the new settlers.

Size cohorts arrived at the nursery from early September to late October. Mortality was substantial and variable and had greater influence than growth on the value of the recruitment potential index (G':Z). No seasonal trends in G':Z were observed and probably most cohorts contributed to recruitment. Caging experiments, while exploratory, may indicate a superior habitat for settlement at the edges of the seagrass by reducing mortality. Finally, hatchery-reared red drum can be used to estimate growth rates of wild counterparts in caging trials but fail to provide information concerning the role of predation with respect to other causes of natural mortality during settlement and subsequent recruitment of red drum to the nursery.

Appendices to chapter 3

A. CORTISOL EXTRACTION PROTOCOL

1. Place 150-200 mg wet-weight tissue in a 2 ml microcentrifuge test tube. Keep the sample on dry-ice.
2. Rinse homogenizer's generator with water and give a final rinse with PBS (0.01 M, pH 7.4).
3. Add 100 µl of PBS and homogenize at medium-high speed for 20 seconds on ice. Rinse generator blade with PBS and collect it in the tube. Rinse homogenizer's generator with water twice and give a final rinse with PBS before proceeding to the next sample.
4. Incubate tubes for 10 minutes in an ultrasonic ice-cold water bath.
5. Bring the volume to 1.5-2 ml with ice cold PBS.
6. Take 500-650 µl to an 11 x 75mm screw-top test tube, add 4 ml Ether and vortex for 2 minutes.
7. Centrifuge at 3000 rpm for 5 minutes at 4 ºC.
8. Freeze the samples and keep them in dry-ice. Decant liquid phase (Ether) into new 11 x 75mm V-bottom centrifuge tube.
9. Thaw the water phase and add 4 ml Ether. Vortex for 2 minutes.
10. Centrifuge at 5000 rpm for 5 minutes at 4 ºC.
11. Freeze the sample and decant liquid phase into same tube as #8. Discard water phase.
12. Dry the pooled supernatants under a stream of nitrogen in a 37-40 ºC dry bath.
13. Concentrate the sample at the bottom of the tube with 100 x 2 µl of Ether. Repeat step #12.

14. Add 150 µL (75 x 2) carbon tetrachloride and vortex for 4 minutes.
15. Add 150 µL PBS-Gel (PBS 0.01 M, pH 7.4, 0.1% Gelatin) and vortex for 2 minutes.
16. Centrifuge at 5000 rpm for 15 minutes at 4 ºC.
17. Take 130 µL of the upper layer to a new 0.5 ml microcentrifuge tube. Discard the bottom layer.
18. Use the extracted sample immediately or store it at –20 ºC until the day of the assay.

B. THYROID HORMONES EXTRACTION PROTOCOL

1. Place 150-200 mg wet-weight tissue in a 2 ml microcentrifuge test tube. Keep the sample on dry-ice.
2. Rinse homogenizer's generator with water and give a final rinse with PBS (0.01 M, pH 7.4).
3. Add 100 µl of PBS and homogenize at medium-high speed for 20 seconds on ice. Rinse generator blade with PBS and collect it in the tube. Rinse homogenizer's generator with water twice and give a final rinse with PBS before proceed to the next sample.
4. Incubate tubes for 10 minutes in an ultrasonic ice-cold bath.
5. Bring the volume to 1.5-2 ml with ice cold PBS.
6. Take 1.0-1.3 ml to an 11 x 75mm screw-top test tube, add 6 ml ice-cold MeOH and vortex for 2 minutes.
7. Centrifuge at 5000 rpm for 15 minutes at 4ºC.
8. Decant supernatant in to new 10 x 75 mm disposable culture tube.
9. Resuspend pellet with 2 ml MeOH. Vortex for 2 minutes. Centrifuge at 5000 rpm for 15 minutes at 4ºC and decant supernatant into same tube as #7. Discard pellet.
10. Dry the pooled supernatants in a centrifuge under vacuum (Speed vac SC100, Savant, NY) overnight.
11. Add 50 µl MeOH and shake for 5 minutes.
12. Add 50 µl barbital buffer (0.1 M Sodium Barbital, 0.1% Gelatin, pH 8.6) and vortex for 1 minute.
13. Add 200 µl chloroform and vortex for 2 minutes.
14. Centrifuge at 5000 rpm for 5 minutes at 4ºC.

15. Take the upper layer to a new 1.5 ml microcentrifuge tube. Discard the bottom layer.

16. Freeze-dry the sample as in #10. Keep the sample dry at –20ºC. On the day of the assay resuspend the dry extract in 220 µl EIA buffer (PBS; 0.1 M, NaCl 0.15 M, 0.01% Tween 20®, 0.1% BSA, pH 7.4)

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Vita

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